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Health Assessment Document for Chloroform External Review Draft. Parts 1 and 2

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Health Assessment Document for Chloroform

Part 1 of 2

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This document represents a comprehensive data base that considers all sources of chloroform in the environment, the likelihood of human exposures and the possible consequences to man and lower organisms from its absorption. This information is integrated into a format that can serve as the basis for qualitative and quantitative risk assessments, while at the same time identifying gaps in our knowledge that limit present evaluative capabilities. Accordingly, it is expected that this document may serve the information needs of many government agencies and private groups that may be involved in decision making and regulatory activities.

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U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
Research Triangle Park, NC 27711

March 1984

PREFACE

The Office of Health and Environmental Assessment has prepared this health assessment to serve as a "source document" for EPA use. This health assessment document was developed for use by the Office of Air Quality Planning and Standards to support decision-making regarding possible regulation of chloroform as a hazardous air pollutant.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated and summary/conclusions have been prepared so that chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and other measures of dose-response relationships are discussed, where appropriate, so that the nature of the adverse health response are placed in perspective with observed environmental levels.

This document will be subjected to a thorough copy editing and proofing following the revision based on the EPA's Scientific Advisory Board review comments.

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The Office of Health and Environmental Assessment (OHEA), U.S. EPA, is responsible for the preparation of this health assessment document. The Environmental Criteria and Assessment Office (ECAO/RTP), OHEA, had the overall responsibility for coordination and the document production effort.

Project Manager

Si Duk Lee, Ph.D.
Environmental Criteria and Assessment Office
U.S. EPA, Research Triangle Park, N.C. 27711
(919) 541-4159

Authors and Reviewers

The principal authors of this document are:

Larry Anderson Ph.D.
Carcinogen Assessment Group
U.S. EPA, Washington, D.C.

David Baylis, M.S.
Carcinogen Assessment Group
U.S., EPA, Washington, D.C.

Chao W. Chen, Ph.D.
Carcinogen Assessment Group
U.S., EPA, Washington, D.C.

Carol Sakai, Ph.D.
Reproductive Effects Assessment Group
U.S., EPA, Washington, D.C.

Sheila Rosenthal, Ph.D.
Reproductive Effects Assessment Group
U.S., EPA, Washington, D.C.

I.W.F. Davidson, Ph.D.
Bowman Gray School of Medicine
Winston Salem, N.C.

D. Anthony Gray, Ph.D.
Syracuse Research Corp.
Syracuse, N.Y.

Sharon B. Wilbur, M.A.
Syracuse Research Corp.
Syracuse, N.Y.

Joan P. Coleman, Ph.D.
Syracuse Research Corp.
Syracuse, N.Y.

The following individuals provided peer-review of this draft or earlier drafts of this document:

U.S. Environmental Protection Agency

Joseph Padgett
Office of Air Quality Planning and Standards
U.S. EPA

Karen Blanchard
Office of Air Quality Planning and Standards
U.S. EPA

Jerry F. Stara, D.V.M.
Office of Health and Environmental Assessment
Environmental Criteria and Assessment Office
U.S. EPA

Lester D. Grant, Ph.D.
Office of Health and Environmental Assessment
Environmental Criteria and Asssessment Office
U.S. EPA

Participating Members of the Carcinogen Assessment Group

Roy E. Albert, M.D. (Chairman)
Elizabeth L. Anderson, Ph.D.
Larry D. Anderson, Ph.D.
Steven Bayard, Ph.D.
David L. Bayliss, M.S.
Chao W. Chen, Ph.D.
Margaret M. L. Chu, Ph.D.
Bernard H. Haberman, D.V.M., M.S.
Charalingayya B. Hiremath, Ph.D.
Robert E. McGaughy, Ph.D.
Dharm W. Singh, D.V.M., Ph.D.
Todd W. Thorslund, Sc.D.

Participating Members of the Reproductive Effects Assessment Group

Peter E. Voytek, Ph.D. (Director)
John R. Fowle, III, Ph.D.
Carol Sakai, Ph.D.
Ernest Jackson, M.D.
K.S. Lavappa, Ph.D.
Sheila Rosenthal, Ph.D.
Vicki Vaughn-Dellarco, Ph.D.

External Peer Reviewers

Dr. Karim Ahmed
Natural Resources Defense Fund
122 E. 42nd Street
New York, N.Y. 10168

Dr. Eula Bingham
Graduate Studies and Research
University of Cincinnati (ML-627)
Cincinnati, Ohio 45221
(513) 475-4532

Dr. James Buss
Chemical Institute of Industrial
Toxicology
Research Triangle Park, N.C. 27709

Dr. I.W.F. Davidson
Wake Forest University
Bowman Gray Medical School
Winston Salem, N.C.

Dr. Larry Fishbein
National Center for Toxicological
Research
Jefferson, Arkansas 72079
(501) 542-4390

Dr. Derek Hodgson
Department of Chemistry
University of North Carolina
Chapel Hill, N.C. 27514

Dr. Marshall Johnson
Thomas Jefferson Medical College
Department of Anatomy
1020 Locust Street
Philadelphia, Pennsylvania 19107

Dr. Trent Lewis
National Institute of Occupational
Safety and Health
26 Columbia Parkway
Cincinnati, Ohio 45226
(513) 684-8394

Dr. Richard Reitz
Dow Chemical, USA
Toxicology Research Laboratory
1803 Building
Midland, Michigan 48640

Dr. Marvin A. Schneiderman
Clement Associates, Incorporated
Arlington, Virginia 22209
(703) 276-7700

Dr. Bernard Schwetz
National Institute of Environmental
Health
Research Triangle Park, NC 27709
(919) 541-7992

Dr. James Selkirk
Oak Ridge National Laboratory
Oakridge, Tennessee 37820
(615) 624-0831

Dr. Samuel Shibko
Food and Drug Administration
Division of Toxicology
200 C Street, S.W.
Washington, D.C. 20204
Telephone:

Dr. Robert Tardiff
1423 Trapline Court
Vienna, Virginia 22180
(703) 276-7700

Dr. Norman M. Trieff
University of Texas Medical Branch
Department of Pathology, UTMB
Galveston, Texas 77550
(409) 761-1895

Dr. Benjamin Van Duuren
Institute of Environmental Medicine
New York University Medical Center
New York, New York 10016
(212) 340-5629

Dr. James Withey
Health and Protection Branch
Department of National Health &
Welfare
Tunney's Pasture
Ottawa, Ontario
CANADA, KIA 0lZ

1. SUMMARY AND CONCLUSIONS

Chloroform is a dense, colorless, volatile liquid used primarily in the production of chlorodifluoromethane (90%) and for export (5%). Non-consumptive uses (5%) include use as a solvent, as a cleaning agent, and as a fumigant ingredient. Direct United States production of chloroform in 1981 was 184 million kg, while indirect production is estimated at 13.2 million kg (≈193 million kg overall). The amount of chloroform emitted to air is estimated to be 7.2 million kg, emissions to water are 2.6 million kg, and emissions to land are 0.6 million kg. Total United States emissions are estimated to be 10.4 million kg.

Chloroform is ubiquitous in the environment, having been found in urban and non-urban locations. The northern hemisphere background average has been determined to be 14 ppt (10^{-12} v/v), while the southern hemisphere has been determined to be <3 ppt. The global average is 8 ppt. For the most part, urban ambient air concentrations remain <1000 ppt, and rural or remote locations can be <10 ppt. There are some notable exceptions, however, the reasons for this are not readily apparent. The highest values reported were in Rutherford, New Jersey, (31,000 ppt) and Niagara Falls, New York (21,611 ppt).

Hydroxyl radical oxidation is the primary atmospheric reaction of chloroform. Based on the rate constant for reaction with chloroform, a half-life of 11.5 weeks is expected. The principal products from this reaction are HCl and CO_2. It has been estimated that roughly 1% of the tropospheric chloroform will diffuse into the stratosphere, based on a lifetime of 0.2 to 0.3 years and a troposphere-to-stratosphere turnover time of 30 years. An EXAMS model of chloroform in water confirms other data that the major removal process for chloroform in water is evaporation.

The best analytical method for detection of chloroform appears to be gas chromatography with electron capture or electrolytic conductivity detector. This gives a detection limit of <5 ppt.

The pharmacokinetics and metabolism of chloroform have been studied in both humans and experimental animals. Chloroform is rapidly and extensively absorbed through the respiratory and gastrointestinal tracts. Absorption through the skin would make a significant contribution to body burden only in instances of contact of the skin with liquid chloroform.

The available data suggest that, for resting human at least 2 hours of exposure are required to reach an apparent body equilibrium with the inhaled chloroform concentration. The percentage of the inhaled chloroform concentration retained in the body at "equilibrium" would be ≈65%, and is independent of the inhaled concentration. The magnitude of chloroform uptake into the body (dose or body burden) is directly proportional to the concentration of chloroform in the inspired air, the duration of exposure, and the respiratory minute volume, and can be estimated by multiplying the percent retention by the total volume of air breathed during exposure and by the exposure concentration.

The absorption of chloroform from the gastrointestinal tract appears to be virtually complete, judging from recovery of unchanged chloroform and metabolites in the exhaled air of humans and in the exhaled air, urine, feces, and carcass of experimental animals. Peak blood levels occurred at ≈1 hour after oral administration of chloroform in olive oil to humans or animals.

After inhalation or ingestion, highest concentrations of chloroform are found in tissues with higher lipid contents. Results from the administration of ^{14}C-labeled chloroform to animals indicate that the distribution of radioactivity (reflecting both chloroform and its metabolites) may be affected by the route of exposure. Oral administration appeared to result in the accumulation of a greater proportion of radioactivity in the liver than did inhalation exposure, but differences in experimental protocols make this interpretation tentative. Sex differences in the distribution of chloroform and its metabolites were found only in mice and not in rats or squirrel monkeys. The kidneys of male mice accumulated strikingly more radioactivity than did those of female mice. Other than the renal accumulation of radioactivity in male mice, the tissue distribution of radioactivity after oral administration was similar in mice, rats, and squirrel monkeys.

Chloroform has been detected in fetal liver. Chloroform would be expected to appear in human milk, because it has been found in cow's milk, cheese, and butter.

Chloroform is metabolized via microsomal cytochrome P-450 oxidation to a reactive intermediate. The primary end product of chloroform metabolism is CO_2, but small amounts of the reactive intermediate bind covalently to tissue macromolecules or conjugate with cysteine and glutathione. Covalent binding of the reactive intermediate to macromolecules is considered to be responsible for the hepato- and nephrotoxicity of chloroform.

The initial product of cytochrome P-450 oxidation of chloroform is trichloromethanol, which spontaneously dehydrochlorinates to produce phosgene. Phosgene is thought to be the toxic reactive intermediate produced during the metabolism of chloroform. Phosgene reacts with water to yield CO_2, with protein to form a covalently bound product, and with cysteine and glutathione. While the liver is the primary site for chloroform metabolism, other tissues, including the kidney, can also metabolize chloroform to CO_2.

Interspecies comparisons of the magnitude of chloroform metabolism have been made only for the oral route. Mice, rats, and squirrel monkeys metabolized 85, 66, and 18%, respectively, of a 60 mg/kg body weight dose of ^{14}C-chloroform to $^{14}CO_2$(measured in the expired air). Most of the remainder was exhaled as unchanged chloroform; small amounts of radioactivity (2 to 8% of the dose) were excreted in the urine and feces. Human subjects (1 or 2/dose) who ingested 0.1, 0.5, and 1.0 g of ^{13}C-chloroform (≃1.4, 7, and 14 mg/kg body weight) metabolized all of the low dose, 50% of the intermediate dose, and only ≃35% of the high dose, judging from the excretion of unchanged chloroform and $^{13}CO_2$ through the lungs. This difference indicates that the fraction of the dose metabolized is dose-dependent.

Regardless of the route of entry into the body, chloroform is excreted unchanged through the lungs and eliminated via metabolism, with the primary stable metabolite, CO_2, also being excreted through the lungs. High concentrations of unchanged chloroform have been found in the bile of squirrel monkeys after oral administration, but not in the urine or feces. The inorganic chloride generated from chloroform metabolism is excreted via the urine.

Decay curves for the pulmonary excretion of unchanged chloroform in humans appear to consist of three exponential components. The terminal component, thought to correspond to elimination from adipose tissue, had a half-time of 36 hours. This long half-time, together with the relatively high levels of chloroform found in adipose tissues of humans known to have been exposed only to ambient levels of the chemical and of animals exposed experimentally, indicates a potential for bioaccumulation.

The adverse health effects of exposure to chloroform include neurological, hepatic, renal, and cardiac effects. These effects have been documented in humans as well as in experimental animals. In addition, studies with animals indicate that chloroform is carcinogenic and may be teratogenic.

Evidence of chloroform's effects on humans has been obtained primarily during the use of this chemical as an inhalation anesthetic. In addition to depression of the central nervous system, chloroform anesthesia was associated with cardiac arhythmias (and some cases of cardiac arrest), hepatic necrosis and fatty degeneration, polyuria, albuminuria, and in cases of severe poisoning, renal tubular necrosis. When used for obstetrical anesthesia, chloroform was likely to produce respiratory depression in the infant. Experimental exposures of humans to chloroform have focused only on subjective responses. Humans exposed experimentally to chloroform for 20 to 30 minutes have reported dizziness, headache, giddiness, and tiredness at concentrations >1000 ppm, and light intoxication at concentrations above 4000 ppm.

Similar symptoms occurred in workers employed in the manufacture of lozenges containing chloroform; exposure concentrations ranged from 20 to 237 ppm, with occasional brief exposure to ≈1000 ppm. Additional complaints were of gastrointestinal distress, and frequent and scalding micturition. The only other report of adverse effects stemming from occupational exposure to chloroform was of enlargement of the liver; this report was compromised by the apparent lack of suitable controls.

Acute inhalation experiments with animals revealed that single exposures to 100 ppm were sufficient to produce mild hepatic effects in mice. The exposure level that would produce mild renal effects is not known, but frank toxic effects occurred in the kidneys of male mice exposed to 5 mg/ℓ (1025 ppm). In subchronic inhalation experiments, histological evidence of mild hepato- and nephrotoxicity occurred in rats with exposures to as low as 25 ppm, 7 hours/day for 6 months. The effects were reversible if exposure was terminated, and did not occur when exposure was limited to 4 hours/day.

Information on the effects of acute and long-term oral exposure to chloroform is available primarily from experiments with animals. Human data are mainly in the form of case reports and involve the abuse of medications containing not only chloroform, but other potentially toxic ingredients as well;

however, a fatal dose of as little as 1/3 ounce was reported. As with inhalation exposure, the primary effects of oral exposure were hepatic and renal damage. Narcosis also occurred with high doses, but this effect was not usually a focus of concern in these experiments. Subchronic and chronic toxicity experiments with rats, mice, and dogs did not clearly establish a no-effect level of exposure for systemic toxicity. Although a dose level of 17 mg/kg/day of chloroform produced no adverse effect in four strains of mice, the lowest dosage tested, 15 mg/kg/day, elevated some clinical chemistry indices of hepatic damage in dogs and appeared to affect a component of the reticuloendothelial system (histiocytes) in their livers.

No controlled studies have been performed to define dose-response thresholds for neurological or cardiac effects of ingested or inhaled chloroform. It is not known whether subtle impairment of neurological or cardiac function might occur at levels as low as or lower than those which affect the liver.

Several substances, which are of interest because of accidental or intentional human exposure, have been shown to modify the systemic toxicity of chloroform, usually by modifying the metabolism of chloroform to the reactive intermediate. Examples of substances that potentiate chloroform-induced toxicity are ethanol, PBBs, ketones and steroids. Factors that appear to protect against toxicity include disulfiram and high carbohydrate diets.

Chloroform appears to have teratogenic potential in laboratory animals when inhaled. Chloroform was selectively more toxic to the fetuses than to the dams when pregnant rats and mice were exposed to the vapor. Delayed fetal development occurred at an exposure level (30 ppm) that produced minimal maternal effects. Embryotoxic effects and low but statistically significant incidences of teratogenic effects occurred at an exposure level that produced mild to moderate maternal effects (i.e., 100 ppm). When chloroform was administered orally (via gavage) to pregnant rats and rabbits, however, toxic effects on fetal development occurred only at dosage levels that produced severe toxic effects in the dams; no teratogenic effects were observed at any dosage level in these animals.

It has been demonstrated that chloroform can be metabolized in vivo and in vitro to a substance(s) (presumably phosgene) that interacts with protein and lipid. However, the sole experiment measuring interaction of metabolically activated chloroform with DNA yielded a negative result. This result was judged as inconclusive, because the specific activity of the $^{14}CHCl_3$ may have been too low.

The majority of the assays for mutagenicity and genotoxicity have also yielded negative results; however, many of these results are inconclusive because of various inadequacies in the experimental protocols used. The major problem is with those bacterial, sister chromatid exchange, and chromosome aberration studies that used reconstituted exogenous activation systems (i.e., S-9 mix). In none of these studies was it shown that chloroform was activated or metabolized by the activation system used. Metabolism of 2-aminoanthracene or vinyl compounds (used as positive controls) is probably an inadequate indication that the activation system can metabolize chloroform because these substances are not halogenated alkanes and are therefore not metabolized like them. A better indication that an activation system is sufficient for metabolism of chloroform may be to show that it metabolizes $^{14}CHCl_3$ to intermediates that bind to macromolecules. A second problem with experimental protocols utilizing exogenous activation systems relates to the possiblity that any reactive metabolic intermediates formed may react with microsomal or membrane lipid or protein before reaching the DNA of the test organism. A third potential problem occurs in those in vitro protocols in which precautions were not taken to prevent escape of volatilized $CHCl_3$.

Studies in which endogenous activation systems were used include those in yeast, in Drosophila (sex-linked recessive lethal), and in mice (bone-marrow micronucleus, sperm head abnormalities and host-mediated assay). The results from several of these studies suggest that chloroform may be a weak mutagen.

In summary, with the present data, no definitive conclusions can be reached concerning the mutagenicity of chloroform. However, there is some indication (from the binding studies and from the mutagenicity tests that utilized endogenous or in vivo metabolism) that chloroform may have the potential to be a weak mutagen. In order to substantiate this, only certain well-designed in vivo mutagenicity studies or studies with organisms possessing endogenous activation systems are recommended.

Chloroform in corn oil administered at maximally and one-half maximally tolerated doses by gavage for 78 weeks produced a statistically significant increase in the incidence of hepatocellular carcinomas in male and female B6C3F1 mice and renal epithelial tumors (malignant and benign) in male Osborne-Mendel rats; a carcinogenic response of female Osborne-Mendel rats to chloroform was not apparent in this study.

A statistically significant increase in the incidence of renal tumors (benign and malignant) was found in another study in male ICI mice treated with chloroform in either toothpaste or arachis oil by gavage for 80 weeks; however, treatment with a gavage dose of chloroform in toothpaste for 80 weeks did not produce a carcinogenic response in female ICI mice and male mice of the CBA, C57BL, and CF/1 strains. A carcinogenic response was not observed in male and female Sprague-Dawley rats given chloroform in toothpaste by gavage for 80 weeks, but early mortality was high in control and treatment groups. Gavage doses of chloroform in toothpaste did not show a carcinogenic effect in male and female beagle dogs treated for 7 years; however, the treatment period was short in relation to the lifespan of the beagle dog. Results of preliminary toxicity tests and the carcinogenicity studies indicate that doses of chloroform in toothpaste given to mice, rats, and dogs in the carcinogenicity studies approached maximally tolerated doses. However, doses of chloroform in toothpaste given to mice and rats were lower than those given in corn oil.

Hepatomas were found in NLC mice given chloroform twice weekly for an unspecified period of time and in female strain A mice given chloroform once every 4 days for a total of 30 doses at a level which produced liver necrosis; however, small numbers of animals were examined in pathology, the duration of studies was below the lifetime of the animals, and no control group of NLC mice was apparent. Although a carcinogenic effect of chloroform was not evident in newborn (C57 x DBA2 - F1) mice given single or multiple subcutaneous doses during the initial 8 days of life and observed for their lifetimes, the dose levels used appeared well below a maximum tolerated dose and the period of treatment after birth was quite short compared to lifetime treatment. Chloroform was ineffective at maximally tolerated and lower doses in a pulmonary adenoma bioassay in Strain A mice. Although an ability of chloroform to promote growth and spread of Lewis lung carcinoma, Erlich ascites, and B16 melanoma cells in mice has been shown, the mechanism by which chloroform produced this effect is uncertain, and the relevance of this study to the evaluation of the carcinogenic potential of chloroform is presently not clear.

There are no epidemiologic studies of cancer and chloroform per se. There appears to be an increased risk of cancer of the bladder, rectum, and large intestine from chlorinated drinking water and, by inference, possibly from chloroform, the predominate contaminant.

In conclusion, evidence that chloroform has carcinogenic activity is based on increased incidences of hepatocellular carcinomas in male and female B6C3F1 mice, renal epithelial tumors in male Osborne-Mendel rats, kidney tumors in male ICI mice, and hepatomas in NLC and female strain A mice. Applying the International Agency for Research on Cancer (IARC) criteria for animal studies, this level of evidence would be sufficient for concluding that chloroform is carcinogenic in experimental animals.

Although there is limited evidence in humans for the carcinogenicity of chlorinated drinking water, and by inference, possibly for chloroform, the human evidence for chloroform itself is insufficient. Considering both human and animal evidence, the overall IARC classification would be Group 2B, meaning that chloroform is probably carcinogenic in humans.

Four data sets that contain sufficient information are used to estimate the carcinogenic potency of chloroform. They are: liver tumors in female mice, liver tumors in male mice, kidney tumors in male rats, and kidney tumors in male mice. The carcinogenic potencies, calculated by the linearized multistage model on the basis of these four data sets, are comparable within an order of magnitude. The two data sets for liver tumors in female and male mice give slightly higher potency values than the two data sets for kidney tumors in male rats and male mice. The geometric mean, q_1^* = 7 x 10-2/(mg/kg/day), of the potencies calculated from liver tumors in male and female mice (the most sensitive species), is taken to represent the carcinogenic potency of chloroform. Based on this potency, the upper-bound estimate of the cancer risk due to 1 µg/m3 of chloroform in air is P = 1 x 10-5. The upper-bound estimate of the cancer risk due to 1 µg/liter in water is P = 2 x 10-6. These estimates appear consistent with the limited epidemiologic data available for humans. The estimated potency of chloroform is in the fourth quartile of 53 suspect carcinogens that have been evaluated by the Carcinogen Assessment Group.

2. INTRODUCTION

The U.S. Environmental Protection Agency is responsible under the authority of various laws for the identification, comprehensive assessment, and as appropriate regulation, of environmental substances which may be of concern to the public health. For example, under section 112 of the Clean Air Act the EPA Administrator is directed to establish standards for any air pollutant (other than those for which national ambient air quality standards are applicable) which, in his judgment, "causes, or contributes to, air pollution which may reasonably be anticipated to result in an increase in mortality or an increase in serious irreversible, or incapacitating reversible, illness."

Within EPA, the Office of Health and Environmental Assessment is responsible for providing scientific assessments of health effects for potentially hazardous air pollutants such as chloroform. These health assessment documents form the scientific basis for subsequent agency actions, including the Administrator's judgment as to whether regulations or standards may be appropriate.

This Health Assessment Document for Chloroform represents a comprehensive data base that considers all sources of chloroform in the environment, the likelihood of human exposures and the possible consequences to man and lower organisms from its absorption. This information is integrated into a format that can serve as the basis for qualitative and quantitative risk assessments, while at the same time identifying gaps in our knowledge that limit present evaluative capabilities. Accordingly, it is expected that this document may serve the information needs of many government agencies and private groups that may be involved in decision making and regulatory activities. (As with all such EPA documents, this preliminary draft is made available to the scientific community and the general public so that comments of interested individuals and organizations may be considered, and the latest scientific evidence incorporated, in the final draft. This draft will also be reviewed by the Environmental Health Advisory Committee of EPA's Science Advisory Board at a subsequently announced public meeting).

3. BACKGROUND INFORMATION

3.1. INTRODUCTION

This section provides background information supportive of the human health effects data presented in subsequent sections. It is not intended to be a comprehensive review of analytical methodology, sources, emissions, air concentrations, or environmental transport and fate information. In order to fulfill this purpose and since the literature concerning chloroform is vast, only a portion of the available literature was included. Those articles included were chosen because of their relevance to the topic at hand and because they were representative of the literature as a whole.

To provide the most complete overview possible, some non-peer reviewed information has been added, from the Chloroform Materials Balance Draft Report by Rem et al. (1982). This is an updated version of the original Level I Materials Balance: Chloroform (Wagner et al., 1980). As described by Wagner et al. (1980):

"A Level I Materials Balance requires the lowest level of effort and involves a survey of readily available information for constructing the materials balance. Ordinarily, many assumptions must be made in accounting for gaps in information; however, all are substantiated to the greatest degree possible. Where possible, the uncertainties in numerical values are given, otherwise they are estimated. Data gaps are identified and recommendations are made for filling them. A Level I Materials Balance relies heavily on the EPA's Chemical Information Division (CID) as a source of data and references involving readily available information. Most Level I Materials Balance are completed within a 3-6 week period; CID literature searches generally require a 2 week period to complete. Thus, the total time required for completion of a Level I materials balance ranges from 5-7 weeks."

Because a greater level of effort went into the 1980 and 1982 Materials Balance reports on chloroform than would normally be devoted to background (non-health effects) information in an EPA health assessment document, information in this chapter is drawn from these reports. However, because such information has not been peer-reviewed and includes some major assumptions, it should not be used to support any regulations or standards regarding risks to public health.

3.2. PHYSICAL AND CHEMICAL PROPERTIES

Chloroform ($CHCl_3$) (CAS registry number 67-66-3) is a member of a family of halogenated saturated aliphatic compounds. Synonyms for chloroform include the following:

- Chloroforme (French)
- Chloroformio (Italian)
- Formyl trichloride
- Methane trichloride
- Methane, trichloror
- Methenyl chloride
- Methenyl trichloride
- Methyl trichloride
- NCI-C02686
- Trichloromethaan (Dutch)
- Trichloroform
- Trichloromethane

Table 3-1 lists important physical properties. Cloroform is a colorless, clear, dense, volatile liquid with an ethereal non-irritating odor (DeShon, 1979). Chloroform is nonflammable; however, when hot chloroform vapors are mixed with alcohol vapors, the mixture burns with a greenish flame. At 25°C and 1 atmosphere, a 1 ppm concentration of chloroform in air is equal to 4.88 mg/m^3.

Chloroform decomposes with prolonged exposure to sunlight regardless of the presence of air (DeShon, 1979). It also decomposes in the dark in the presence

TABLE 3-1

Physical Properties of Chloroform[a]

Molecular weight	119.38
Melting point (°C)	-63.2
Boiling Point	61.3
Water-chloroform azeotrope (°C)	56.1
Specific gravity (25/4°C)	1.48069
Vapor density (101kPa, 0°C, kg/m^3)	4.36

Vapor pressure

°C	kPa	torr
-30	1.33	10.0
-20	2.61	19.6
-10	4.63	34.7
0	8.13	61.0
10	13.40	100.5
20	21.28	159.6
30	32.80	246.0
40	48.85	366.4

Solubility in water

°C	g/kg H_2O
0	10.62
10	8.95
20	8.22
30	7.76

Log octanol/water partition coefficient[b]	1.97

Conversion factors at 25°C and 1 atm[c]
1 ppm $CHCl_3$ in air equals 4.88 mg/m^3
1 mg/m^3 $CHCl_3$ in air equals 0.205 ppm

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[a] Source: DeShon, 1979

[b] Hansch and Leo, 1979

[c] Calculated

of air. The principal decomposition products include phosgene, hydrogen chloride, chlorine, carbon dioxide, and water. Ozone causes chloroform to decompose rapidly.

Chloroform forms a hydrate in water at 0°C ($CHCl_3 \cdot 18H_2O$, CAS registry number 67922-19-4); the hexagonal crystal decomposes at 1.6°C (DeShon, 1979). Chloroform is chemically stable to water, having a hydrolysis half-life of 3100 years in neutral water at 25°C (Mabey and Mill, 1978); the half-life of chloroform in air from hydroxyl radical reactions is 78 days (Hampson, 1980).

The hydrogen atom on chloroform can be removed in the presence of warm alkali metal hydoxide to form a trichloromethyl anion (DeShon, 1979). This anion can condense with carbonyl compounds. Both wet and dry chloroform will react with aluminum, zinc, and iron.

Small amounts of ethanol are used to stabilize chloroform from oxidation during storage (DeShon, 1979).

3.3. SAMPLING AND ANALYSIS

3.3.1. Chloroform in Air. Chloroform in air can be analyzed by a number of methods; however, the method of Singh et al. (1980) appears to be substantially free of artifact problems and completely quantitative. In this method, an air sample in a stainless steel canister at 32 psig is connected to a preconcentration trap consisting of a 4" x 1/16" ID stainless steel tube containing glass beads, glass wool, or 3% SE-30 on acid washed 100/120 mesh chromosorb W. The sampling line and trap, maintained at 90°C, are flushed with air from the canister; then the trap is immersed in liquid O_2 and air is passed through the trap, the initial and final pressure being noted (usually between 30 and 20 psig) on a high-precision pressure guage. The ideal gas law can be used to estimate the volume of air passed through the trap. The contents of the trap are desorbed onto a chromatography column by backflushing it with an inert gas while holding the trap at boiling water temperature. An Ascarite trap may be inserted before the chromatography column to remove water. Suitable columns include 20% SP-2100 and 0.1% CW-1500 on Supelcoport (100/120 mesh, 6' x 1/8" stainless steel) and 20% DC-200 on Supelcoport (80/100 mesh, 33' x 1/8" Ni). Both columns can be operated at 45°C with a carrier gas flow of 40 mℓ/min on the former column and 25 mℓ/min on the latter. An electron capture detector operating at 325°C was found to be optimum. It should be noted that the above authors found Tenax to be unsuitable for air analyses because of the presence of artifacts in the spectrum from oxidation of the Tenax monomer. In addition, when Tenax is used as a sorbent, safe sampling volumes (i.e., that volume of air which, if sampled over a variety of circumstances, will not cause significant breakthrough) should be adhered to. Brown and Purnell (1979) determined the safe volume for chloroform per gram Tenax to be 9.3 ℓ (flow rate 5-600 mℓ/min; $CHCl_3$ conc. <250 mg/m^3 temp. up to 20°C) with a safe desorption temperature of 90°C.

The detection limits of this method were not specified and are dependent on the volume of air sampled. Analyses as low as 16 ppt have been reported using this method (Singh et al., 1980).

3.3.2. Chloroform in Water. Chloroform in water can be analyzed by the purge-and-trap method (Method 502.1) as recommended by the Environmental Monitoring and Support Laboratory of the U.S. EPA (1981a). In this method, an inert gas is bubbled through 5 mℓ of water at a rate of 40 mℓ/minute for 11 minutes, allowing the purgable organic compounds to partition into the gas. The gas is passed through a column containing Tenax GC at 22°C, which traps most of the organics removed from the water. The Tenax column is then heated rapidly to 130°C and backflushed with helium (20-60 mℓ/minute, 4 minutes) to desorb the trapped organics. The effluent of the Tenax column is passed into an analytical gas chromatography column packed with 1% SP-1000 on Carbopack-B (60/80 mesh, 8'x 0.1"

I.D.) maintained at 40°C. The column is then temperature programmed starting at 45°C for 3 minutes and increasing at 8°C/minute until 220°C is reached; it is then held there for 15 minutes or until all compounds have eluted. A halogen-specific detector (or GC-MS) having a sensitivity of 0.10 µg/ℓ with a relative standard deviation of <10% must be used.

3.3.3 Chloroform in Blood. Chloroform in blood can be analyzed by using a modified purge-and-trap method (Pellizzari et al., 1979). This method involves diluting an aliquot of whole blood (with anticoagulant) to ≈50 mℓ with prepurged, distilled water. The mixture is placed in a 100 mℓ 3-neck round bottom flask along with a teflon-lined magnetic stirring bar. The necks of the flask are equipped with a helium inlet, a Tenax trap, and a thermometer. The Tenax trap is a 10 cm x 1.5 cm i.d. glass tube containing pre-extracted (Soxhlet, methanol, 24 hrs) and conditioned (270°C, 30 mℓ/min helium flow, 20 min) 35/60 mesh Tenax (≈1.6 g, 6 cm). The sample is then heated to 50°C and purged with a helium flow rate of 25 mℓ/min for 90 min. Analysis can be performed as indicated in Section 3.3.2.

3.3.4. Chloroform in Urine. Chloroform in urine can be analyzed by using an apparatus identical to the one described in Section 3.3.3, using 25 mℓ of urine diluted to 50 mℓ instead of blood.

3.3.5. Chloroform in Tissue. Chloroform in tissue can be analyzed by using an apparatus identical to the one described in Section 3.3.3, using 5 g of tissue diluted to 50 mℓ and macerated in an ice bath instead of blood. The purge time is reduced to 30 minutes.

3.4. EMISSIONS FROM PRODUCTION AND USE

3.4.1. Emissions from Production.

3.4.1.1. DIRECT PRODUCTION -- Chloroform is produced commercially in the United States by two methods, chlorination of methane and chlorination of methyl chloride produced from methanol and hydrogen chloride (Wagner et al., 1980; DeShon, 1979). The chemistry is summarized by the following reactions:

Methane Chlorination

$$4CH_4 + 10Cl_2 \longrightarrow CH_3Cl + CH_2Cl_2 + CHCl_3 + CCl_4 + 10HCl$$

Methanol Hydrochlorination

$$HCl + CH_3OH \xrightarrow[280 - 350°C]{\text{Catalyst}} CH_3Cl + H_2O$$

$$3CH_3Cl + 6Cl_2 \longrightarrow CH_2Cl_2 + CHCl_3 + CCl_4 + 6HCl$$

The methanol process accounts for 74% of capacity, while methane accounts for 26% of capacity (SRI International, 1983).

In the chlorination of methane, natural gas is directly chlorinated in the gas phase with chlorine at 485-510°C (Anthony, 1979). The product mixture contains all chlorinated methanes, which are removed by scrubbing and separated by fractional distillation.

In the second process, gaseous methanol and HCl are combined over a hot catalyst to form methyl chloride (Ahlstrom and Steele, 1979). The methyl chloride is then chlorinated with chlorine to produce CH_2Cl_2, $CHCl_3$, and CCl_4 (DeShon, 1979). The chlorination conditions for both processes can be adjusted to optimize chloroform production.

United States production is carried out by five manufacturers at seven sites summarized in Table 3-2.

The annual production of chloroform in the United States has risen from 35 million kg (77 million lbs) in 1960 (DeShon, 1979) to >184 million kg (405 million lbs) in 1981 (USITC, 1982) with few declines.

TABLE 3-2

Chloroform Producers, Production Sites, and Capacities*

Producer	Production Site	Capacity Millions of kg (Millions of lbs)	Process
Diamond Shamrock	Belle, WV	18(40)	Methanol
Dow Chemical	Freeport, TX	45(100)	Methane
	Plaquemine, LA	45(100)	Methanol
Linden Chemicals and Plastics, Inc.	Moundsville, WV	14(30)	Methanol
Stauffer Chemical Co.	Louisville, KY	34(75)	Methanol
Vulcan Materials Co.	Geismar, LA	27(60)	Methanol
	Wichita, KA	50(110)	Methanol and Methane
TOTAL		233(515)	

*Source: SRI International, 1983.

3.4.1.1.1. Chloroform Emissions from the Methane Chlorination Process--Rem et al. (1982) reported that air emissions could come from process vents, in-process and product storage tanks, liquid waste streams, secondary emissions (handling and disposal of process wastes), and fugitive emissions from leaks in process valves, pumps, compressors and pressure relief valves.

The emission factors they calculated were based on a typical methane chlorination facility as reported by U.S. EPA (1980a) having a total chloromethane capacity of 2×10^5 metric tons (441×10^6 lbs), operating continuously (8760 hr/yr), and having a product mix of 20% CH_3Cl, 45% CH_2Cl_2, 25% $CHCl_3$, and 10% CCl_4. The emission factor for the uncontrolled recycle methane inert gas purge vent for the above plant (0.014 kg/metric ton) was calculated from an hourly $CHCl_3$ emission rate of 0.071 kg/hr reported by Dow Chemical Company for a 46,000 metric ton/yr facility assuming continuous (8760 hr/yr) operation (Beale, n.d.). The uncontrolled emission factor for the distillation area emergency inert gas vent (0.032 kg/metric ton) was calculated from an emission factor for volatile organic compounds (VOC) of 0.20 kg/metric ton of total chloromethane production and composition data showing chloroform to be 4% of VOC (U.S. EPA, 1980a). In-process and product storage emissions (0.91 to 0.80 kg/metric ton, depending on controls) were calculated from emission equations for breathing and working losses from AP-61 (U.S. EPA, 1981b) assuming tanks to be half-full, have 95% emission controls when present, and a 12°C diurnal temperature variation (U.S. EPA, 1980b). Rem et al. (1982) calculated the total chloroform emissions to air to be 70.2 metric tons (155×10^3 lbs) by multiplying the appropriate factors by plant capacity use after including secondary emissions (0.21 kg/metric ton) and fugitive emissions (5.5 kg/hr).

Releases of chloroform to water come from scrubbers, neutralizers, and cooling water (Rem et al., 1982). Based on a 300 ppm $CHCl_3$ content in total wastewater discharges averaging 68 ℓ/min and assuming that 90% would volatilize, Rem et al. (1982) calculated a release factor of 0.023 kg/metric ton. They then added to this the emission from indirect contact cooling water (100 ppm, 5800 ℓ cooling water/metric ton $CHCl_3$, 90% evaporation) to calculate a release rate of 3.3 metric tons of $CHCl_3$ (7.3×10^3 lbs) per year to water.

No quantifiable data were available to Rem et al. (1982) regarding release of chloroform to land from methane chlorination.

3.4.1.1.2. Chlorination Emissions from the Methanol Hydrochlorination-Methyl Chloride Chlorination Process -- Chloroform emissions to air come from process vents, in-process and product storage tank emissions, and fugitive emissions from leaks in valves, pumps, compressors, and pressure relief valves (Rem et al., 1982). Rem et al. (1982) used an uncontrolled emission factor reported by Vulcan Materials Company for process vents (Hobbs, 1978), and assumed continuous (8,760 hr/year) operation, and controls sufficient to reduce emissions 80% to obtain the emission factor for controlled process vents (0.003 kg/metric ton for controlled; 0.015 kg/metric ton for uncontrolled). Storage emissions (0.176 kg/metric ton for controlled, 0.88 kg/metric ton for uncontrolled) were calculated from Hobbs (1978) and from emission equations for breathing and working losses from AP-42 (U.S. EPA, 1981b), assuming tanks to be half-full, have 80% emission reduction controls, and a 12°C diurnal temperature variation (U.S. EPA, 1980b). Fugitive emission factors (3.32 kg/hr for uncontrolled; 1.08 kg/hr for controlled) for volatile organic compounds were used (U.S. EPA, 1980c) along with a control factor of 67.5% based on leak detection and repair (U.S. EPA, 1980b). Emission rates were then calculated to be 196 metric tons (432×10^3 lbs) based on plant capacity, capacity use (66%), the level of emission controls used at each plant, and continuous operation (8,760 hr/year).

Rem et al. (1982) assumed that releases of chloroform to water from methyl chloride chlorination resulted from contamination of cooling water and from spent acid and spent caustic streams. For cooling water contamination, they assumed minor spills and leaks resulted in contamination of 100 mg chloroform/ℓ cooling water, that 5,800 ℓ of cooling water per metric ton of chloroform produced was consumed, and that 90% of the chloroform evaporated. For spent acid and spent caustic streams they assumed that 0.04 kg of chloroform are released for every metric ton of chloromethane produced. They further stated that this release factor was not considered to be very reliable; however, in the absence of better data they used this factor to approximate emissions. In addition, they assumed that 1/3 of the chloromethane production consists of chloroform and that 90% of the released chloroform evaporates. Using these assumptions, Rem et al. (1982) calculated that 0.070 kg of chloroform is released to water per metric ton of chloroform produced, or 8.0 metric tons (17.6 x 10^3 lbs) of chloroform were released to water based on 1980 production levels.

Rem et al. (1982) reported that the bottoms from chloroform distillation in the methyl chloride chlorination process are the feed for carbon tetrachloride and perchloroethylene production, and that during their production a residue is formed that contains chloroform. This residue is landfilled or deep-well injected. This represents the only known release of chloroform from carbon-tetrachloride/perchloroethylene production.

Rem et al. (1982) assumed that 1.02 kg of residue is produced/metric ton of chloroform from methyl chloride production (see Wagner et al., 1980). They further assumed (as did Wagner et al., 1980) that 18.4% of the residue is chloroform and that 25% is landfilled. This results in a release factor of 0.047 kg chloroform per metric ton of chloroform produced, or 5.4 metric tons (12 x 10^3 lbs) based on 1980 production.

3.4.1.1.3. Summary of Direct Production -- Direct production emits some 283 metric tons into the environment on an annual basis. Greater than 95% (266.2 metric tons) of this is emitted into the air. Direct production accounts for ≈3% of all environmentally released chloroform, and ≈3.6% of all chloroform released to air. Table 3-3 summarizes chloroform discharges from direct production.

3.4.1.2. INDIRECT PRODUCTION

3.4.1.2.1. Chloroform Formation During Ethylene Dichloride Production -- Ethylene dichloride (EDC) is produced by two methods, direct chlorination and oxychlorination, and is used principally for vinyl chloride monomer (VCM) production. A combination of the two methods is used by most VCM production facilities in a process known as the balanced process since the HCl from the dehydrochlorination of EDC is used to produce more EDC from ethylene, the major products of the overall reaction being VCM and H_2O.

Direct Chlorination

$$CH_2 = CH_2 + Cl_2 \longrightarrow CH_2ClCH_2Cl$$

Oxychlorination

$$2\ HCl + 1/2\ O_2 \xrightarrow{\text{catalyst}} Cl_2 + H_2O$$

$$CH_2 = CH_2 + Cl_2 \longrightarrow CH_2ClCH_2Cl$$

Balanced Process

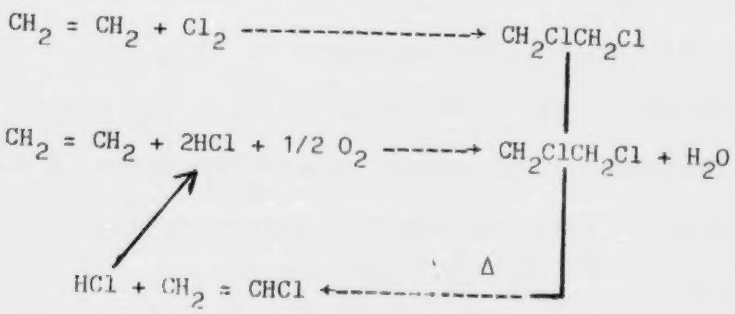

TABLE 3-3

Chloroform Discharges from Direct Sources*

	Environment Release (metric tons/year)			
Source	Air	Water	Land	Total
Methyl Chloride Chlorination	196	8	5.4	209.4
Methane Chlorination	70.2	3.3	---	73.5
Total:	266.2	11.3	5.4	282.9

*Source: Rem et al. (1982)

Chloroform is formed as a byproduct during EDC manufacture. Rem et al. (1982) estimated chloroform emissions to air from EDC production based on emission factors developed by the EPA during field studies of domestic EDC production facilities (see U.S. EPA, 1980d). Domestic production facilities are listed in Table 3-4. Emission sources (e.g., process vents, fugitive emissions, storage) for each plant, plant capacity, capacity utilization (56%), and control technology were all combined with the emission factors to determine the overall chloroform emissions at the current level of control. According to their calculations, chloroform emissions to the atmosphere are ≃760 metric tons/year (1,675 x 10^3 lbs/year).

Chloroform releases to water may occur during the discharge of wastewater from the process; however, the amount of chloroform present is unknown.

Chloroform releases to land from EDC production reportedly occur when the light ends from EDC distillation are landfilled (Rem et al., 1982). An estimated 217 metric tons (478 x 10^3 lbs) were landfilled in 1980.

3.4.1.2.2. Chlorination of Drinking Water -- Chloroform in drinking water arises when humic substances or methyl ketones (e.g., acetone) in water react with a hypochlorite anion (Stevens et al., 1976; NAS, 1978). Hypochlorite is the principal reactant in chlorinated water above pH 5. Chloroform is produced by the haloform reaction outlined below:

$$R\text{-}COCH_3 + 3OCl^- \longrightarrow RCOCCl_3 + 3OH^-$$

$$R\text{-}COCCl_3 + OH^- \longrightarrow RCOO^- + CHCl_3$$

Rem et al. (1982) used the data from the National Organics Reconnaissance Survey (NORS) (Symons et al., 1975) and the National Organics Monitoring Survey (NOMS) (U.S. EPA, n.d.) to estimate the concentration of chloroform in drinking

TABLE 3-4

Ethylene Dichloride Producers, Production Sites and Capacities[a]

Producer	Production Site	Capacity millions of Kg	(millions of lbs)
Alantic Richfield Co. ARCO Chem. Co., div.	Port Arthur, TX	204	(450)
Borden Inc. Borden Chem. Div. Petrochems. Div.	Geismar, LA	231	(510)
Dow Chemical. U.S.A.	Freeport, TX	726	(1600)
	Oyster Creek, TX	476	(1050)
	Plaquemine, LA	862	(1900)
E.I. du Pont de Nemours and Co., Inc. Conoco Inc., subsid. Conoco Chems. Co. Div.	Lake Charles, LA	524	(1155)
Ethyl Corp. Chems. Group	Baton Rouge, LA	318	(700)
	Pasadena, TX	102	(225)
Formosa Plastics Corp. U.S.A.	Baton Rouge, LA	249	(550)
	Point Comfort, TX	386	(850)
Georgia-Pacific Corp. Chem. Div.	Plaquemine, LA	748	(1650)
The BF Goodrich Co. BF Goodrich Chem. Group	Deer Park, TX	145	(320)
	La Porte, TX	719	(1585)
Convent Chem. Corp., subsid.	Calvert City, KY	454	(1000)
	Convent, LA	363	(800)
PPG Indust., Inc. Chems. Group Chem. Div.	Lake Charles, LA	1225	(2700)

TABLE 3-4 (cont.)

Producer	Production Site	Capacity millions of Kg	(millions of lbs)
Shell Chem. Co.	Deer Park, TX	635	(1400)
	Norco, LA	544	(1200)
Union Carbide Corp. Ethylene Oxide Derivatives Div.	Taft, LA	68[b]	(150)
	Texas City, TX	68	(150)
Vulcan Materials Co. Vulcan Chems., div.	Geismar, LA	159	(350)
	TOTAL	9,206	20,295

[a] Source: SRI International, 1983

[b] Captive use only

water. These surveys provided information on chloroform concentrations in 137 U.S. cities. To determine the amount of chloroform generated, the authors multiplied the volume of water treated by each city by the chloroform concentration in the drinking water. The amount of chloroform generated by each city was then summed and divided by the volume of water generated to give a weighted average concentration of 41 μg/ℓ. This was then multiplied by the estimated volume of water chlorinated annually (4.6×10^{13} ℓ/year) to yield the amount in the U.S. (1,900 metric tons, 4.2×10^6 lbs). Rem et al. (1982) stressed that this value probably represents a minimum since NORS was conducted during the winter (hence, chloroform levels were low) and NOMS samples were iced when taken (hence, may be lower than if allowed full contact time). Thus, the actual value may be higher than this estimate.

3.4.1.2.3. Chlorination of Municipal Sewage -- Chlorination of municipal sewage results in increased chloroform concentrations (NAS, 1978). Municipal wastewater generally contains a lower concentration of chloroform precursors (humic materials) than do ambient waters; therefore, the amount of chloroform generated from wastewater chlorination is smaller (Wagner et al., 1980). Rem et al. (1982) calculated chloroform production from wastewater treatment based on analyses of the secondary effluent from 28 municipal plants published by the EPA (U.S. EPA, 1979a). These analyses showed that the average chloroform concentration increased by 9 μg/ℓ, from 5 to 14 μg/ℓ. Rem et al. (1982) assumed that all municipal wastewater was chlorinated and multiplied the average concentration increase by the municipal wastewater flow (9.7×10^{10} ℓ/day) listed in U.S. EPA (1981c). By this method, 320 metric tons (0.7×10^6 lbs) was calculated to be produced annually.

3.4.1.2.4. Chlorination of Cooling Waters -- Cooling water used in electric power generating plants is treated with chlorine as a biocide to prevent fouling intake screens and condensers in both once-through and closed cycle systems (U.S. EPA, 1980e). Rem et al. (1982) calculated chloroform production based on the size of the average power plant (hence, the volume of water required), the type of cooling system used (once-through or recirculating), and the fact that 65% of all power plants chlorinate cooling water. They calculate that 72 metric tons of chloroform (160 x 10^3 lbs) are discharged directly into water by once-through systems and 190 metric tons of chloroform (420 x 10^3 lbs) are emitted into the air by recirculating systems. A total of 262 metric tons of chloroform (580 x 10^3 lbs) are produced annually from cooling water chlorination.

3.4.1.2.5. Chlorination in the Pulp and Paper Industry -- Pulp and paper mills emit more chloroform to the environment than any other single source. Chloroform is produced during the bleaching of wood pulp, a process that whitens the final paper product. Rem et al. (1982) based their estimation on information contained in a number of documents concerned with the pulp and paper industry (U.S. EPA, 1980f; NCASI, 1977; Metcalf and Eddy Inc., 1972; TAPPI, 1963). From the operating conditions, analytical data, and production steps detailed in these documents, Rem et al. (1982) determined the quantity of chloroform produced for each of nine different types of mills for which monitoring data existed and applied these values to all mills for which no values from sampling existed. The authors determined that chloroform is emitted at three different stages: into the air during the bleaching process itself, into the air during the detention time in wastewater treatment plants, and into the water from treatment plant effluent. The amount of chloroform produced annually was calculated to be 128 metric tons (282 x 10^3 lbs) released to air during bleaching operations, 3,985 metric tons (8.78 x 10^6 lbs) released to air from wastewater detention (4,113 metric tons to air), and 298 metric tons (657 x 10^3 lbs) discharged into water from treatment plants (4,411 metric tons or 9.72 x 10^6 lbs total).

3.4.1.2.6. Chloroform Production from Combustion of Leaded Gasoline -- Chloroforom has been reported to be a component of automobile exhaust (Harsch et al., 1977). Its presence is reportedly the result of using ethylene dichloride and ethylene dibromide as lead scavengers in leaded gasoline (Lowenbach and Schlesinger Associates, 1979). Rem et al. (1982) cite other sources which state that chloroform is not formed during the combustion of leaded fuel containing ethylene dichloride. The Emissions Testing and Characterization Branch of EPA's Environmental Sciences Research Laboratory measured chlorocarbon emissions from automobiles using leaded gasoline and found no chloroform. Rem et al. (1982) then reason that even if chloroform were present in automobile exhaust, the decrease in the usage of leaded gasoline will decrease the amount of chloroform produced.

The authors cite an EPA report (U.S. EPA, 1982a) which states that leaded gasoline consumption is expected to drop by >75%, from 34×10^9 gallons to 8.3×10^9 gallons/year. Rem et al. (1982) used the estimate of Wagner et al. (1980) that 1% of the ethylene dichloride in gasoline would be converted to chloroform, and the new lead phase-down regulations (U.S. EPA, 1982b) to calculate an annual emission rate of 180 metric tons (397×10^3 lbs) in 1983 and 44 metric tons (97×10^3 lbs) by 1990.

3.4.1.2.7. Chloroform Formation During Atmospheric Trichloroethylene Decomposition -- Trichloroethylene is a major industrial solvent used principally for vapor degreasing of fabricated metal parts (66%) (Chemical Marketing Reporter, 1981), and the majority of each year's production is used for replacement of evaporative loss to the environment (Rem et al., 1982). The postulated formation of chloroform during the atmospheric decomposition of trichloroethylene is based on laboratory experiments in which trichloroethylene, NO_2, H_2O, and a hydrocarbon mixture were irradiated with light having the intensity and spectral distribution of the lower troposphere (U.S. EPA, 1976). Dichloroacetylchloride, phosgene, chloroform, and HCl were detected as products. Rem et al. (1982) did not describe the method they used to determine the amount of chloroform produced from this reaction; however, they estimated 780 metric tons of chloroform are produced annually.

3.4.1.2.8. Miscellaneous Indirect Source -- Wagner et al. (1980) have listed a number of other indirect sources of chloroform that are difficult if not impossible to quantify. These sources are: chlorination wastewaters from the textile industry, the food processing industry, breweries, combustion of tobacco products treated with chlorination pesticides, thermal decomposition of plastics, biological production in red marine algae, and the reaction of chlorinated pollutants with humic substances in natural waters.

3.4.1.2.9. Summary of Indirect Production -- Chloroform is produced and emitted into the environment from a variety of indirect sources. These sources account for ≈84% of all chloroform air emissions, 98% of water discharges, and 36% of all land discharges (84.6% of all environmental releases).

The environmental discharges of chloroform from indirect sources are summarized in Table 3-5.

3.4.2. Emissions from Use. Chloroform is used consumptively for the production of chlorodifluoromethane or Fluorocarbon-22 (accounts for 90% of domestic 1982 production, 60% for refrigerants, 30% for fluoropolymer) and for exports (3% in 1982) (Anonymous, 1983). Non-consumptive uses include its use as an extraction solvent; as a solvent for penicillin, alkaloids, vitamins, flavors, lacquers, floor polishes, artificial silk manufacture, resins, fats, greases, gums, waxes, adhesives, oils, and rubber; as a dry cleaning agent; as an intermediate in pesticide and dye manufacture; and as a fumigant ingredient (Rem et al., 1982; DeShon, 1979; Merck Index, 1976). The great majority of chloroform used non-

TABLE 3-5

Chloroform Discharges from Indirect Sources[a]

Source	Environmental Release (metric tons/year)			
	Air	Water	Land	Total
Pulp and Paper Mills	4113	298	0	4411
Drinking Water Chlorination	0	1900	0	1900
Ethylene Dichloride Manufacture	760	---[b]	217	977
Trichloroethylene Photodegradation	780	0	0	780
Municipal Wastewater Chlorination	0	320	0	320
Cooling Water Chlorination	190	72	0	262
Automobile Exhaust	180	0	0	180
TOTAL	6023	2590	217	8830

[a] Source: Rem et al., 1982

[b] minor releases possible

consumptively is emitted into the environment since (except for expansions) the chloroform purchased for these uses is make-up solvent used to replace that amount not recovered from processes (Wagner et al., 1980).

3.4.2.1. EMISSIONS FROM PHARMACEUTICAL MANUFACTURING -- Chloroform is used as an extraction solvent during the manufacture of some antibiotics and steriods, and during the manufacture of certain other biological and natural pharmaceuticals (Rem et al., 1982). It is also used as a chemical intermediate. Based on a Pharmaceutical Manufacturing Association (PMA) Survey, Rem et al. (1982) reported that ≈1000 metric tons of chloroform are released into the environment (no year was specified; the PMA report was dated 1978). The distribution was as follows: 57.0% (570 metric tons) to air, 4.6% (46 metric tons) to water, and 38.4% (384 metric tons) to land.

3.4.2.2. EMISSIONS FROM FLUOROCARBON-22 PRODUCTION -- The single largest use of chloroform is for Fluorocarbon-22 production (Chlorodifluoromethane, $CHClF_2$). Fluorocarbon-22 producers and production sites are listed in Table 3-6. Chloroform release to the environment can occur from process emissions, fugitive emissions, and storage emissions (Rem et al., 1982). Rem et al. (1982) reported that the first source listed does not represent a significant source of chloroform emissions based on the design of Fluorocarbon-22 production facilities and the process description.

Storage emission estimates were based on U.S. EPA (1980g) for chloroform feedstock storage in fixed roof tanks. Rem et al. (1982) reported that the Allied Chemical facility at El Segundo, California, uses a control system that results in complete capture of chloroform vapors. Du Pont (and all others by assumption) use refrigerated condensers that reduce the uncontrolled emission factor of 2.5 kg/metric ton by 66%. Fugitive emissions from leaks in valves, pumps, compressors, and relief valves was estimated to result in an uncontrolled

TABLE 3-6

Chlorodifluoromethane Producers and Production Sites*

Producer	Production Site
Allied Corp. Allied Chem. Co.	Baton Rouge, LA Danville, IL El Segundo, CA
E.I. du Pont de Nemours and Co., Inc. Petrochems. Dept. Freon Products Div.	Deepwater, NJ Louisville, KY
Pennwalt Corp. Chems. Group Fluorochemicals Div.	Calvert City, KY

*Source: SRI International, 1983

emission rate of 0.75 kg/metric ton. Total emissions to air were calculated by Rem et al. (1982) to be 139 metric tons/year by multiplying the emission factors by 1980 Fluorocarbon-22 production (97,500 metric tons).

No estimate was made for emissions to wastewater because of a lack of data. Emissions to land were based on the reported practice of landfilling spent catalyst by Allied. Rem et al. (1982) assumed a catalyst contamination level of 10% and a total emission of 2.0 metric tons/year.

3.4.2.3. EMISSIONS FROM HYPALON® MANUFACTURE -- Hypalon® is a chemically resistant synthetic rubber made by substituting chloride and sulfonyl groups onto polyethylene. The process involves dissolving polyethylene in chloroform followed by reaction with chlorine and sulfur dioxide. Based on a Du Pont report, Rem et al. (1982) estimated that 54.9 metric tons of chloroform were emitted into the air from Hypalon® manufacture in 1980, based on an emission inventory conducted by the Texas Air Control Board, Austin, Texas. No information was available for water or land emissions.

3.4.2.4. CHLOROFORM EMISSIONS FROM GRAIN FUMIGATION -- Chloroform is a registered pesticide for use on certain insects that commonly infest stored raw bulk grains and is present in only one product (Rem et al., 1982). This product, Chlorofume® FC.30 Grain Fumigant (Reg. No. 5382-15), marketed by Vulcan Materials Company, contains 72.2% chloroform, 20.4% carbon disulfide, and 7.4% ethylene dibromide. Originally registered in 1968, the EPA issued a "Notice of Presumption Against Continued Registration of a Pesticide Product -- Chloroform (Trichloromethane)" in 1976 because of oncogenic effects in rats and mice. Continued study resulted in returning it to the normal registration process (U.S. EPA, 1982c). Based on a personal communication with D. Lindsay of Vulcan Materials, Rem et al. (1980) estimated that between 10,000 and 12,000 gallons of chloroform/year were used in grain fumigants in the United States. Vulcan reported 1981 sales of 7,000 gallons of Chlorofume® in 1981 or 5054 gallons (19,131 ℓ). Based on its density, 28,400 kg (28.4 metric tons) was released to the environment (air) in this way.

3.4.2.5. CHLOROFORM LOSSES FROM LOADING AND TRANSPORTATION -- Rem et al. (1982) estimated chloroform losses from loading ships, barges, tank cars, and tank trucks. The method was based on the degree of chloroform saturation of the air expelled from tanks during filling, temperature, vapor pressure, control efficiency, and filling methods as described by U.S. EPA (1979b) and Environment Reporter (1982). The U.S. mode of transportation was taken from Sax (1981) as follows: rail, 40.3%; barge, 47.8%; and truck, 11.9%. Loading losses were calculated to be 40.9 metric tons (90,200 lbs).

Transit losses result from temperature and barometric pressure changes. The losses were assumed to be the same for barges, tank trucks, and rail cars and were estimated from the following equation:

$$L_T = 0.1\ PW$$

where LT= transit loss, lb/week - 10^3 gal transported

P= true vapor pressure of transported liquid, psia

W= density of condensed vapors lb/gal

No reference or justification for the use of the formula was presented. By this method, and assuming 1 week transit time, Rem et al. (1982) calculated that 49.2 metric tons (0.11×10^6 lbs) chloroform were lost to the air using 1980 production values.

3.4.2.6. MISCELLANEOUS USE EMISSIONS -- Rem et al. (1982) cited the previous materials balance (Wagner et al., 1980) in predicting the emissions from chloroform contamination of methyl chloride, methylene chloride, and carbon tetrachloride. Chloroform is present to some extent in these products since they are all made by the same process. Assuming a contamination level of 7.5 ppm, 17.5 ppm and 150 ppm for methyl chloride, methylene chloride, and carbon tetrachloride, respectively, Rem et al. (1982) estimate that releases to air, land and water would be 9.8, 0.6, and 0.15 metric tons, respectively.

Chloroform is also used in a variety of products (see Section 3.4.2) and as a general solvent. Rem et al. (1982) estimate that, while these uses are generally declining, laboratory uses in particular may account for 8.5% of production or 14,200 metric tons of chloroform. Rem et al. (1982), however, estimated the range of uncertainty to be ± 50%.

3.4.2.7. SUMMARY OF CHLOROFORM DISCHARGES FROM USE -- Chloroform discharges from manufacturing facilities that use chloroform as a process ingredient account for 12% of all chloroform emissions to air, 1.8% of water discharges, and 63% of all land discharges, or 12.7% of chloroform discharges to all media. Table 3-7 summarizes chloroform discharges to all media.

3.4.3. Summary. Chloroform is produced by direct and indirect processes. Direct production accounts for 184 million kg annually, while indirect production accounts for ≈8.8 million kg annually.

Direct production of chloroform and processes associated with its use (i.e., Fluorocarbon-22 production, Hypalon® manufacture, loading and transit losses, grain fumigation, pharmaceutical use) emit some 1.6 million kg to the environment. Virtually all of the indirectly produced chloroform is emitted into the environment; the total amount of chloroform emitted is ≈10.4 million kg. This represents ≈5.6% of direct production. The relative source contributions from all quantifiable sources are listed in Table 3-8.

3.5. AMBIENT AIR CONCENTRATIONS

Monitoring data for a number of U.S. and world locations are presented in Table 3-9. For the most part, ambient concentrations remain <1000 ppt (1 ppt =

TABLE 3-7

Chloroform Discharges from Use[a]

Source	Environmental Release (metric tons/year) Air	Water	Land	Total
Pharmaceuticals	570	46	384	1000
Chlorodifluoromethane Manufacture	139	--[b]	2	141
Loading and Transit Losses	90.1	0	0	90.1
Hypalon® Manufacture	54.9	--[b]	--[b]	54.9
Grain Fumigation	28.4	0	0	28.4
Secondary Product Contamination	9.8	0.6	0.2	10.6
Total	892.2	46.6	386.2	1325.0

[a] Source: Rem et al., 1982

[b] minor releases possible

TABLE 3-8

Relative Source Contribution for Chloroform[a]

	Environmental Release (metric tons/year)							
Source	Air	% of Total[c]	Water	% of Total[c]	Land	% of Total[c]	Total	% of Total[c]
Pulp and Paper Mills	4113	39	298	2.9	0	0	4411	42
Drinking Water Chlorination	0	0	1900	18	0	0	1900	18
Pharmaceuticals	570	5.5	46	0.4	384	3.7	1000	10
Ethylene Dichloride Manufacuture	760	7.3	---[b]	----	217	2.1	977	9.4
Trichloroethylene Photodegradation	780	7.5	0	0	0	0	780	7.5
Municipal Wastewater Chlorination	0	0	320	3.1	0	0	320	3.1
Cooling Water Chlorination	190	1.8	72	0.7	0	0	262	2.5
Methyl Chloride Chlorination	196	1.9	8	0.1	5.4	0.1	209.4	2.0
Automobile exhaust	180	1.7	0	0	0	0	180	1.7
Chlorodifluoromethane Manufacture	139	1.3	----	----	2	0.02	141	1.4
Loading and Transit Losses	90.1	0.9	0	0	0	0	90.1	0.9
Methane Chlorination	70.2	0.7	3.3	0.03	----	----	73.5	0.7
Hypalon® Manufacture	54.9	0.5	----	----	----	----	54.9	0.5
Grain Fumigation	28.4	0.3	0	0	0	0	28.4	0.3
Secondary Product Contamination	9.8	0.1	0.6	0.006	0.2	0.002	10.6	0.1
Laboratory Usage[d]	----	----	----	----	----	----	----	----
TOTAL	7181.4	68.8	2647.9	25.4	608.6	5.8	10,438[c]	

[a] Source: Rem et al. (1982)

[b] dashed lines indicate minor releases possible

[c] values are rounded

[d] not included because of uncertainty

TABLE 3-9

Ambient Levels of Chloroform

Location	Type of Site	Date	Analytical Method	Concentration (ppt, v/v)			Reference
				Max.	Min.	Average	
Alabama							
Tuscaloosa	urban	2/77	GC-ECD	3000	100	800	Holzer et al., 1977
Talladega Forest	rural	2/77	GC-ECD	200	NR	100	Holzer et al., 1977
Arizona							
Phoenix	urban	4-5/79	GC-coulometry	514.0	27.1	111.4	Singh et al., 1981
California							
Stanford Hills	clean[a]	11/75	GC-ECD	217	12	33	Singh et al., 1979
Point Reyes	clean marine	12/75	GC-ECD	114	15	37	Singh et al., 1979
Los Angeles	urban	4-5/76	GC-ECD	724	23	102	Singh et al., 1979
Palm Springs	urban-suburban	5/76	GC-ECD	616	20	99	Singh et al., 1979
Yosemite	remote-high altitude	5/76	GC-ECD	24	12	17	Singh et al., 1979
Mill Valley	clean marine[a]	1/77	GC-ECD	36	4	25	Singh et al., 1979
Riverside	urban-suburban	4-5/77	GC-ECD	310	24	25	Singh et al., 1979
Badger Pass	remote-high altitude	5/77	GC-ECD	38	2	16	Singh et al., 1979
Point Arena	clean marine	5/77	GC-ECD	42	8	20	Singh et al., 1979
Point Arena	clean marine	8-9/78	GC-ECD	48	12	18	Singh et al., 1979
Los Angeles	urban	4/79	GC-ECD	223.5	24.3	88.2	Singh et al., 1981
Oakland	urban	6-7/79	GC-ECD	60.1	13.1	32.1	Singh et al., 1981
Delaware							
Delaware City	NS	7/74	GC-ECD	<10	<10	NR	Lillian et al., 1975a
Kansas							
Jetmore	remote-continental	6/78	GC-ECD	34	9	16	Singh et al., 1979
Maryland							
Baltimore	urban	7/74	GC-ECD	<10	<10	NR	Lillian et al., 1975a
Montana							
Western Montana 1979	remote	3/76	GC-MS	NR	NR	9	Cronn and Harsch, 1979
Nebraska							
Reese River	remote-high altitude	5/77	GC-ECD	19	6	13	Singh et al., 1979
New Jersey							
Rutherford	urban	1978	GC-MS	31,000	NR	4600	Bozzelli and Kebbekus, 1979
Newark	urban	1978	GC-MS	7500	NR	3900	Bozzelli and Kebbekus, 1979

TABLE 3-9 (cont.)

Location	Type of Site	Date	Analytical Method	Concentration (ppt, v/v) Max.	Min.	Average	Reference
Piscataway	urban	1978	GC-MS	2900	NR	2200	Bozzelli and Kebbekus, 1979
Somerset (county)	urban	1978	GC-MS	11,000	NR	5000	Bozzelli and Kebbekus, 1979
Bridgewater Township	rural	1978	GC-MS	NR	NR	ND^b	Bozzelli and Kebbekus, 1979
Bound Brook	urban	3/76	GC-MS	NR	NR	854	Pellizzari, 1977
Patterson	urban	3/76	GC-MS	NR	NR	768	Pellizzari, 1977
Clifton	urban	3/76	GC-MS	NR	NR	1700	Pellizzari, 1977
Fords	urban	3/76	GC-MS	NR	NR	3422	Pellizzari, 1977
Newark	urban	3/76	GC-MS	NR	NR	7582	Pellizzari, 1977
Passaic	urban	3/76	GC-MS	NR	NR	854	Pellizzari, 1977
Hoboken	urban	3/76	GC-MS	NR	NR	427	Pellizzari, 1977
Seagrit	urban	6/74	GC-ECD	60	<10	40	Lillian et al., 1975a
Seagrit	urban	6/75	GC-ECD	50	5	35	Lillian et al., 1975b
Sandy Hook	urban	7/74	GC-ECD	63	<10	30	Lillian et al., 1975a
Sandy Hook	urban	7/75	GC-ECD	55	10	25	Lillian et al., 1975b
Bayonne	urban	7/75	GC-ECD	15,000	<10	1030	Lillian et al., 1975a
New York							
Staten Island	urban	3/76	GC-MS	NR	NR	4268	Pellizzari, 1977
New York City	urban	6/74	GC-ECD	480	<10	160	Lillian et al., 1975a
New York City	urban	6/75	GC-ECD	450	10	200	Lillian et al., 1975b
White Face Mountain	remote	9/74	GC-ECD	250	<10	9	Lillian et al., 1975a
White Face Mountain	remote	9/75	GC-ECD	350	6	8	Lillian et al., 1975b
Niagara Falls	urban	NS	GC-MS	21,611	215	NR	Pellizzari et al., 1979
Ohio							
Wilmington	Air Force Base	7/74	GC-ECD	4800	<10	340	Lillian et al., 1975a
Wilmington	Air Force Base	7/75	GC-ECD	5000	20	480	Lillian et al., 1975a
Texas							
Houston	urban	6-7/77	GC-MS	11,034	Trace	NR	Pellizzari et al., 1979

TABLE 3-9 (cont.)

Location	Type of Site	Date	Analytical Method	Concentration (ppt, v/v) Max.	Min.	Average	Reference
Washington							
Pullman	rural	12/74 to 2/75	GC-MS	NR	NR	20	Grimsrud and Rasmussen, 1975
Pullman	rural	11/75	GC-ECD	NR	NR	43	Rasmussen et al., 1977
England							
Liverpool/Manchester	suburban	NS	GC-ECD	8^c	3^c	NR	Pearson and McConnell, 1975
Organochlorine manufacturer	urban	NS	GC-ECD	40^c	$<10.1^c$	NR	Pearson and McConnell, 1975
Moel Faman, Flintshire	urban	NS	GC-ECD	0.4^c	$<0.1^c$	NR	Pearson and McConnell, 1975
Rannoch Moor, Argyllshire	urban	NS	GC	0.5^c	0.1^c	NR	Murray and Riley, 1973
Rural areas	rural	NS	GC	1.2	0.82	0.82	Murray and Riley, 1973
Ireland							
Cork	urban	1974	GC-ECD	NR	NR	26.5	Cox et al., 1976
Japan							
Kobe	NS	NS	GC-ECD	9400	300		Okuno et al., 1974
Atlantic Ocean							
Northeast Atlantic (Cape Blanc to Lands End) 34°19'N 13°32'W to 49°54'N 05°54'W		7-8/72	GC	0.96	0.14	0.35	Murray and Riley, 1973

[a] Subject Urban Transport

[b] Detection Limits 10 ppt

[c] ppb by mass

NR = Not reported; NS = Not specified

ND = Not detected

10^{-12}, v/v), and some <10 ppt. There are notable exceptions, however, although the reasons for this are not readily apparent.

Singh (1977) and Singh et al. (1979) have determined northern and southern hemisphere background concentrations as well as a global average. The hemispheric values are 14 ppt for the northern hemisphere and <3 ppt for the southern hemisphere. This difference in hemispheric values suggests that the oceans are not a significant source of chloroform, but rather, that chloroform, for the most part, is anthropogenic. The global average concentration determined by Singh et al. (1979) is 8 ppt.

An interesting point not presented in Table 3-9 is that chloroform concentrations above an inversion layer are significantly lower than concentrations below it. In Wilmington, OH, above an inversion layer, the chloroform concentration was <10 ppt, whereas below it the concentration was 120 ppt (Lillian et al., 1975a).

3.6. ATMOSPHERIC REACTIVITY

The principal atmospheric reactant responsible for the removal of chloroform is probably the hydroxyl radical (Atkinson et al., 1979; Graedel, 1978; Altshuller, 1980; Singh, 1977; Crutzen and Fishman, 1977). Hydroxyl radicals are formed in the lower atmosphere in two ways, first, by the photodissociation of ozone ($\lambda < 310$) into O (^1D) atoms (Atkinson et al., 1979). These go on to react with either water, hydrogen or methane to form hydroxyl radicals. The second important source of hydroxyl radicals is the reaction of hydroperoxyl radicals with nitric oxide.

Hydroxyl radical reactions probably follow the course outlined below (Graedel, 1978):

$$CHCl_3 + HO \longrightarrow \cdot CCl_3 + H_2O$$

$$\cdot CCl_3 + O_2 \longrightarrow \cdot O_2CCl_3$$

$$\cdot O_2CCl_3 + NO \longrightarrow \cdot OCCl_3 + NO_2$$

$$\cdot OCCl_3 \longrightarrow COCl_2 \text{ (phosgene)} + Cl\cdot$$

$$COCl_2 + H_2O \longrightarrow CO_2 + 2\,HCl$$

Pearson and McConnell (1975) found HCl and CO_2 as the only products of chloroform irradiation with UV (λ >290nm) light. The half-life reported by these workers (23 weeks) was of the same order of magnitude as that calculated from the hydroxyl radical rate constant (11.5 weeks) (Singh et al., 1981).

Chloroform will not react photolytically in the trosposphere; the UV cutoff for chloroform is 175 nm (i.e., it will not absorb light >175 nm). Callahan et al. (1979) calculated that roughly 1% of the tropospheric chloroform would diffuse eventually into the stratosphere, based on a lifetime of 0.2-0.3 years and a troposphere-to-stratosphere turnover time of 30 years.

3.7. ECOLOGICAL EFFECTS/ENVIRONMENTAL PERSISTENCE

3.7.1. Ecological Effects.

3.7.1.1. TERRESTRIAL -- Data on the terrestrial ecological effects of chloroform are not available. Significant effects are not expected because chloroform is quite volatile and does not accumulate in terrestrial (or aquatic) environments, and is diluted rapidly and degraded to low concentrations in the troposphere (NAS, 1978). Conceivably, acute effects on wildlife can occur in the vicinity of major chloroform spills, but significant chronic effects from long-term exposure to low ambient levels is unlikely.

3.7.1.2. AQUATIC -- The toxicity of chloroform to aquatic organisms has been reviewed by the U.S. EPA (1980h). As summarized in Table 3-10, two freshwater fish (rainbow trout, bluegill) and one invertebrate (*Daphnia magna*) species have been acutely tested under standard conditions; LC_{50} concentrations ranged from 28,900 to 115,000 μg/ℓ (Bentley et al., 1975; U.S. EPA, 1978a), and

TABLE 3-10

Acute and Chronic Effects of Chloroform on Aquatic Organisms[a]

Species	Duration	Concentration ($\mu g/\ell$)	Method	Effect	Reference
Cladoceran *Daphnia magna*	48-hr	28,900	S,U[b]	LC$_{50}$	U.S. EPA, 1978a
Rainbow trout *Salmo gairdneri*	96-hr	66,800	S,U	LC$_{50}$	Bentley et al., 1975
Rainbow trout *Salmo gairdneri*	96-hr	43,800	S,U	LC$_{50}$	Bentley et al., 1975
Bluegill *Lepomis macrochirus*	96-hr	115,000	S,U	LC$_{50}$	Bentley et al., 1975
Bluegill *Lepomis macrochirus*	96-hr	100,000	S,U	LC$_{50}$	Bentley et al., 1975
Pink shrimp *Penaeus duorarum*[c]	96-hr	81,500	S,U	LC$_{50}$	Bentley et al., 1975
Orangespotted sunfish *Lepomis humilis*	1-hr	106,890 to 152,700	NS	death	Clayberg, 1917
Goldfish *Carassius auratus*	30 to 60 min	97,000 to 167,000	NS	50% anesthetized	Cherkin and Catchpool, 1964
Threespine stickleback *Gasterosteus aculeatus*	90-min	207,648[d]	NS	anesthesia with recovery	Jones, 1947a

TABLE 3-10 (cont.)

Species	Duration	Concentration (µg/ℓ)	Method	Effect	Reference
Ninespine stickleback Pungitius pungitius	NS	148,320 to 296,640	NS	Avoidance	Jones, 1947b
Rainbow trout (embryo-larval) Salmo gairdneri	27 days[f]	2,030	F,M[e]	LC_{50} at 50 mg/ℓ hardness	Birge et al., 1979
Rainbow trout (embryo-larval) Salmo gairdneri	27 days[f]	1,240	F,M[e]	LC_{50} at 200 mg/ℓ hardness	Birge et al., 1979
Rainbow trout (embryo) Salmo gairdneri	23 days	10,600	F,M[e]	40% teratogenesis	Birge et al., 1979

[a] Source: U.S. EPA, 1980

[b] Static test, unmeasured concentration

[c] Saltwater species

[d] Corrected from vol/vol to µg/ℓ

[e] Flow-through test, measured concentration

[f] Exposures began within 20 minutes of fertilization and ended 8 days after hatching.

hr = hour; min = minutes; NS = Not stated the trout was more sensitive than the bluegill. With stickleback, goldfish, and orangespotted sunfish, anesthetization or death occurred after exposure to 97,000 to 207,000 μg/ℓ chloroform for 30 to 90 minutes (Clayberg, 1917; Jones, 1947a; Cherkin and Catchpool, 1964). Only one test has been conducted with chloroform and saltwater organisms; the 96-hour LC_{50} for the pink shrimp was 81,500 μg/ℓ (Bentley et al., 1975).

Embryo-larval tests with rainbow trout at 2 levels of hardness provided 27-day LC_{50} values of 2030 and 1240 μg/ℓ (Birge et al., 1979). There was a 40% incidence of teratogenesis in the embryos at hatching (23 day exposure at 10,600 μg/ℓ).

Bluegills bioconcentrated radiolabeled chloroform by a factor of 6 after a 14-day exposure, and the tissue half-life was <1 day (U.S. EPA, 1978a). This degree of bioconcentration and short biological half-life suggest that chloroform residues would not be an environmental hazard to consumers of aquatic life (U.S. EPA, 1980h).

3.7.2. Environmental Persistence. A number of researchers have reported the dominance of hydroxyl radical oxidations in the fate of chloroform in the atmosphere (see Section 3.6). Singh et al. (1981) calculated an atmospheric residence time for chloroform based on the NASA reviewed rate constant reported by Hampson (1980). They reported a 116-day (16.6-week) residence time for a hydroxyl radical concentration of 10^6 molecules/cm^3. This compares well to the observed 33-week lifetime of chloroform in a sunlit flask (Pearson and McConnell, 1975). This lifetime was based on experiments conducted in northwest England, which receives less intense sunlight than most of the U.S., and may account for its longevity.

According to recent hydroxyl radical measurements, tropospheric ambient air concentrations range from $\simeq 10^6$ to 10^7 molecules/mℓ (Atkinson et al., 1979);

models of the troposphere have suggested a concentration ranging between 2 to 6 x 10^5 molecules/mℓ (Crutzen and Fishman, 1977; Singh, 1977).

Table 3-11 summarizes the literature values for k_{OH}, the temperature of measurement, and the calculated lifetime based on the indicated hydroxyl radical concentration. If a hydroxyl radical concentration of 2 x 10^6 molecules/cm^3 (typical for summer months; winter concentrations are lower) (Singh et al., 1981) is assumed, most of the lifetimes calculated from the rate constants range between 0.2 to 0.5 years (69 to 181 days, 122 average).

Mabey and Mill (1978) critically reviewed hydrolysis data available in the literature. They determined that chloroform had a hydrolysis half-life of >3,000 years at pH 7 and 298 K. This is based on a base hydrolysis rate of 0.602 x 10^{-4} and a OH^- concentration of 10^{-7} in neutral water.

Dilling et al. (1975) and Dilling (1977) determined the volatilization half-life of chloroform from water. For a 1 ppm chloroform solution stirred at 200 rpm, the time for 50% removal was 21.5 minutes (average); 90% removal was accomplished in 71 minutes. The addition of dry granular bentonite clay, dolomitic limestone, or peatmoss had little effect on the evaporation rate. The rapid volatilization of chloroform was seen also by Jensen and Rosenberg (1975), who reported that 0.1-1.0 ppm solutions of chloroform in partly open sunlit aquaria lost 50-60% of the chloroform in 8 days as opposed to only 5% in closed aquaria. Pearson and McConnell (1975) suggested that the presence of chloroform in ambient waters may be from aerial transport and washout.

An EXAMS model of the fate of chloroform in a pond, a river, and an oligotrophic lake and eutrophic lake revealed the dominant process in all cases to be volatilization. Input parameters included hydrolysis (2.5 x 10^{-9} hr^{-1}), octanol/water partition coefficient (91), vapor pressure (150.5 torr at 20°C), solubility (8200 ppm), Henry's Law Constant (2.88 x 10^{-3}), reaeration rate ratio

TABLE 3-11

Values for k_{OH}

$k_{OH} \times 10^{14}$ $cm^3 molecule^{-1} sec^{-1}$	K	[OH] $\times 10^{-5}$	Lifetime (years)	Reference
6.51	265	4	1.2	Singh et al., 1979
10.1	296			Howard and Evenson, 1976
16.8	298	10	0.19	Cox et al., 1976
11.4	298			Davis et al., 1976[a]
6.4	265	9	0.56	
7.4	273			Hampson, 1980[b]
10	298			

[a] $(4.69 \pm 0.71) \times 10^{-12} \exp - (2254 \pm 214/RT)$

[b] Evaluated by NASA

(0.583), alkoxy radical rate constant ($0.7\ M^{-1}\ hr^{-1}$) ($RO\cdot = 10^{14}\ M$), and a stream loading of 1 g/hr. No photochemical or bacterial degradation parameters were entered since chloroform has no UV absorbence >290 nm, and virtually no bacterial degradation occurs with chloroform (Pearson and McConnell, 1975; Bouever et al., 1981). Table 3-12 summarizes the EXAMS model generated fate of chloroform.

3.8. EXISTING CRITERIA, STANDARDS, AND GUIDELINES

3.8.1. Air. The Occupational Safety and Health Administration (OSHA) currently limits occupational exposure to chloroform to a ceiling level of 50 ppm (40 CFR 1910.1000). This ceiling level is not to be exceeded in the workplace at any time. To protect against mild central nervous system depression, irritant effects, and fetal abnormalities (which were considered to occur at lower exposure levels than those causing liver injury), the National Institute for Occupational Safety and Health (NIOSH) recommended in 1974 that exposure to chloroform be limited to 10 ppm as a Time-Weighted Average (TWA) exposure for up to a 10-hour workday, 40 hour workweek. A ceiling level of 50 ppm was proposed for any 10-minute period (NIOSH, 1974). NIOSH lowered the recommended criterion to 2 ppm TWA in 1976 (NIOSH, 1977) in response to a positive NCI carcinogenesis bioassay (NCI, 1976). NIOSH recommended that exposure to halogenated anesthetic agents, including chloroform, be limited to 2 ppm because this is the lowest detectable level using the recommended sampling and analysis techniques, and not because a safe level of airborne exposure could be defined.

On the basis of recent reports of carcinogenicity and embryotoxicity, the American Conference of Governmental Industrial Hygienists (ACGIH) currently classifies chloroform as an Industrial Substance Suspect of Carcinogenic Potential for Man (ACGIH, 1981). The ACGIH recommends a Threshold Limit Value (TLV) of 10 ppm and a 15-minute Short-Term Exposure Limit (STEL) TWA of 50 ppm for chloroform.

TABLE 3-12

Summary of EXAMS Models of the Fate of Chloroform[a]

	River	Pond	Oligotrophic Lake	Eutrophic Lake
Maximum total concentrations in water column (mg/ℓ)	9.92×10^{-7}	2.50×10^{-3}	1.33×10^{-4}	1.26×10^{-4}
Maximum concentration in sediments (dissolved in pore water, mg/ℓ)	9.85×10^{-7}	1.36×10^{-3}	5.82×10^{-6}	4.57×10^{-6}
Maximum concentration in Bios				
Plankton (μg/g)	2.58×10^{-5}	6.49×10^{-2}	3.45×10^{-3}	3.27×10^{-3}
Benthos (μg/g)	2.56×10^{-5}	3.53×10^{-2}	1.51×10^{-4}	1.19×10^{-4}
Maximum total concentration in sediment (mg/kg, dry weight)	3.19×10^{-6}	6.50×10^{-3}	2.83×10^{-5}	9.35×10^{-6}
Total steady accumulation (kg)	9.15×10^{-4}	5.43×10^{-2}	0.33	0.3
% in water column	96.93	91.9	99.95	99.94
% in sediments	3.07	8.1	0.05	0.06
Disposition				
chemical transformation (%)	0.00	0.00	0.00	0.00
biotransformation (%)	0.00	0.00	0.00	0.00
volatilization (%)	1.74	93.35	94.98	95.57
Volatilization half-life	36 hours	40 hours	10 days	9 days
exported (%)	98.26	6.65	5.02	4.43
export half-life	0.65 hours	566 hours	192 days	196 days
Mass Flux from Volatilization (kg/hr)	1.74×10^{-5}	9.33×10^{-4}	2.28×10^{-3}	2.29×10^{-2}
Self-Purification Time	37 hours	31 days	65 days	56 days

[a] Based on load of 1.00 g/hr

Foreign industrial air standards for chloroform include (Utidjian, 1976): Bulgaria, 10 ppm; Czechoslovakia, 10 ppm (50 ppm for brief exposures); Finland, 50 ppm; Hungary, 4 ppm (20 ppm for brief exposures); Japan, 50 ppm; Poland, 10 ppm; Rumania, 10 ppm; Yugoslavia, 50 ppm; West Germany, 10 ppm (Utidijian, 1976).

3.8.2. Water. As discussed in the Ambient Water Quality Criteria Document for chloroform (U.S. EPA, 1980h), the EPA has proposed an amendment that would add to the National Interim Primary Drinking Water Regulations a section on the control of organic halogenated chemical contaminants. The proposed limit for total trihalomethanes in drinking water, which includes chloroform as the major constituent, is 100 µg/ℓ. Although some estimates of cancer risk were performed, this limit was set primarily on the basis of technological and economic feasibility, and initially will apply only to water supplies serving >75,000 consumers. The basis and purpose of this regulation are discussed in a report that was prepared by the Office of Drinking Water (U.S. EPA, 1978b).

The U.S. EPA (1980h) recently derived cancer-based ambient water criteria for chloroform. Since zero level concentrations of chloroform will never be attainable in chlorine-treated water, levels that may result in incremental increases of cancer risk over the lifetime were estimated at risks of 1×10^{-5}, 10^{-6}, and 10^{-7}. The corresponding recommended criteria, which were derived with the tumor incidence data from the NCI bioassay with female mice (NCI, 1976), are 1.90, 0.19, and 0.019 µg/ℓ, respectively, if exposure is assumed to be from the consumption of drinking water and fish and shellfish products and at 157 µg/ℓ, 15.7 µg/ℓ, and 1.57 µg/ℓ, respectively, if exposure is assumed to be from the consumption of aquatic organisms only.

3.8.3. Food. Chloroform has been approved by the Food and Drug Administration (FDA) as a component of articles intended for use in contact with food (i.e., an indirect food additive). The use of chloroform in the food industry is summarized as follows:

Component of adhesives	21 CFR 175.105
Adjuvant substance required in the production of polycarbonate resins	21 CFR 177.1580

Chloroform also has been exempted from the requirement of tolerance when used as a solvent in pesticide formulations that are applied to growing crops (40 CFR 180.1001), or when used as a fumigant after harvest for barley, corn, oats, popcorn, rice, rye, sorghum (milo), or wheat (40 CFR 180.1009).

3.8.4. Drugs and Cosmetics. The positive NCI carcinogenicity bioassay of chloroform (NCI, 1976) has prompted the FDA to restrict the use of chloroform in drug (21 CFR 310-513) and cosmetic (21 CFR 700.18) products.

3.9. RELATIVE SOURCE CONTRIBUTIONS

The sum of all the environmental releases of chloroform from all sources listed in Section 3.4.3 amounts to a total of 10,438 metric tons. All sources are summarized in Table 3-8 with the percent of the total emissions. Total emissions from all sources constitute about 5.6% of production (184,000 metric tons). Table 3-8 does not include estimated emissions from laboratory use. Rem et al. (1982) suggested that these are potentially large but gave no numerical estimate.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 1980. Documentation of the Threshold Limit Values. 4th ed. Cincinnati, OH. pp. 90-91.

ACGIH (American Conference of Governmental Industrial Hygienists). 1981. Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1981. Cincinnati, OH. pp. 13, 42.

Ahlstrom, R.C., Jr. and J.M. Steele. 1979. Methylchloride. In: Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed., M. Grayson and D. Eckroth, eds. New York: John Wiley and Sons, Inc. vol. 5. pp. 677-685.

Altshuller. 1980. Lifetimes of organic molecules in the troposphere and lower stratosphere. In: Advances in Environmental Science and Technology. vol. 10. Pitts and Metcalf, eds. New York: John Wiley and Sons, Inc. p. 181-219.

Anonymous. 1983. Chemical Profile. Chloroform. Chemical Marketing Reporter, June 25, 1979, p. 54.

Anthony, T. 1979. Methylene chloride. In: Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed., M. Grayson and D. Eckroth, eds., New York: John Wiley and Sons, Inc. vol. 5. p. 686-693.

Atkinson, R., K.R. Darmall, A.C. Lloyd, A.M. Winer and J.N. Pitts, Jr. 1979. Kinetics and mechanisms of the reactions of the hydroxyl radical with organic compounds in the gas phase. In: Ado. Photochem. 11: 375-488.

Beale, J. (n.d.). Letter from J. Beale, Dow Chemical U.S.A., Midland, Michigan, to L. Evans, Emissions Standards and Engineering Division, U.S. EPA concerning Dow facility at Freeport, TX. (Cited in Rem et al., 1982).

Bentley, R.E., et al. 1975. Acute toxicity of chloroform to bluegill (Lepomis macrochirus), rainbow trout, (Salmo gairdneri), and pink shrimp (Penaeus durorarum). Contract No. WA-6-99-1414-B. U.S. Environmental Protection Agency (Cited in U.S. EPA, 1980h.)

Birge, W.J. et al. 1979. Toxicity of organic chemicals to embryo-larval stages of fish. EPA-560-79-007. U.S. Environmental Protection Agency. (Cited in U.S. EPA, 1980h.)

Bouever, E.J., B. Rittman and P.L. McCarty. 1981. Anaerobic degradation of halogenated 1- and 2-carbon organic compounds. Environ. Sci. Technol. 15: 596-599.

Bozzelli, J.W. and B.B. Kebbekus. 1979. Analysis of selected volatile organic substances in ambient air. State of New Jersey Department of Environmental Protection.

Brown, R.H. and C.J. Purnell. 1979. Collection and analysis of trace organic vapour pollutants in ambient atmospheres. The performance of a Tenax-GC adsorbent tube. J. Chromatog. 178: 79-90.

Callahan, M.A., M.W. Slimak, N.W. Gabel, I.P. May, C.F. Fowler, J.R. Freed, P. Jennings, R.L. Durfee, F.C. Whitmore, B. Maestri, W.R. Mabey, B.R. Holt and C. Gould. 1979. Water related fate of 129 priority pollutants, vol. II. U.S. EPA - 440/4-79-029B.

Chemical Marketing Reporter. 1981. Chemical Profile: Trichloroethylene. April 6, 1981.

Cherkin, A. and J.F. Catchpool. 1964. Temperature dependence of anesthesia in goldfish. Science. 144: 1460. (Cited in U.S. EPA, 1980h.)

Clayberg, H.D. 1917. Effects of ether and chloroform on certain fishes. Biol. Bull. 32: 234. (Cited in U.S. EPA, 1980h.)

Cox, R.A., R.G. Derwent, A.E.J. Eggleton and J.E. Lovelock. 1976. Photochemical oxidation of halocarbons in the troposphere. Atmosph. Environ. 10: 305-308.

Cronn, D.R. and D.E. Harsch. 1979. Determination of atmospheric halocarbon concentrations by gas chromatography-mass spectrometry. Anal. Letters. 12: 1489-1496.

Crutzen, P.J. and J. Fishman. 1977. Average concentrations of OH in the troposphere and budgets of CH_4, CO, H_2, and CH_3CCl_3. Geophys. Res. Lett. 4: 321-324.

Davis, D.D., G. Machado, B. Conaway, Y. Oh and B. Watson. 1976. A temperature dependent kinetics study of the reaction of OH with CH_3Cl, $CHCl_3$, and CH_3Br. Jour. Chem. Phys. 65: 1268-1274.

DeShon, H.D. 1979. Chloroform. In: Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed., M. Grayson and D. Eckroth, eds. New York: John Wiley and Sons, Inc. vol 5. pp. 693-703.

Dilling, W.L. 1977. Interphase transfer processes. II. Evaporation rates of chloromethanes, ethanes, ethylenes, propanes, and propylenes from dilute aqueous solutions. Comparisons with theoretical predictions. Environ. Sci. Technol. 11: 405-409.

Dilling, W.L., N.B. Tefertiller and G.J. Kallos. 1975. Evaporation rates and reactivities of methylene chloride, chloroform, 1,1,1-trichloroethane, trichloroethylene, tetrachlorocthylene, and other chlorinated compounds in dilute aqueous solutions. Environ. Sci. Technol. 9: 833-838.

Environmental Reporter. 1982. State Air Laws. Bureau of National Affairs. Washington, DC. (Cited in Rem et al., 1982).

Graedel, T.E. 1978. Chemical compounds in the atmosphere. Academic Press, NY. p. 331, 334.

Grimsrud, E.P. and R.A. Rasmussen. 1975. Survey and analysis of halocarbons in the atmosphere by gas chromatography-mass spectometry. Atmos. Environ. 9:1014-1017.

Hampson, R.F. 1980. Chemical, kinetic, and photochemical data sheets for atmospheric reactions. Washington, D.C. Department of Transportation Report FAA-EE-80-17.

Harsch, D.E., R.A. Rasmussen, and D. Pierotti. 1977. Identification of a potential source of chloroform in urban air. Chemosphere. 11: 769-775.

Harsch, D.E., D.R. Cronn and W.R. Slater. 1979. Expanded list of halogenated hydrocarbons measureable in ambient air. Jour. Air Pollut. Control Assoc. 29: 975-976.

Harsch, C. and A.J. Leo. 1979. Substituent constants for correlation analysis in chemistry and biology. New York: John Wiley and Sons. p. 172-173.

Hobbs, F.D. 1978. Trip Report for Vulcan Materials Company, Geismar, Louisiana. Prepared for U.S. Environmental Protection Agency. Emissions Standards and Engineering Division, Research Triangle Park, NC. (Cited in Rem et al., 1982).

Holzer, G., H. Shanfield, A. Zlatkis, W. Bertsch, P. Juarez, M. Mayfield and H.M. Liebich. 1977. Collection and analysis of trace organic emissions from natural sources. Jour. Chromatog. 142: 755-764.

Howard, C.J. and K.M. Evenson. 1976. Rate constants for the reactions of hydrozyl with methane and fluorine, chlorine, and bromine substituted methanes at 296° Kelvin. Jour. Chem. Phys. 64: 197-202.

Jensen, S. and R. Rosenberg. 1975. Degradibility of some chlorinated aliphatic hydrocarbons in sea water and sterilized water. water res. 9: 659-661.

Jones, J.R.E. 1947a. The oxygen consumption of Gasterosteus aculeatus L. to toxic solutions. Exp. Biol. 23: 298. (Cited in U.S. EPA, 1980h.)

Jones, J.R.E. 1947b. The reactions of Pygosteus gungitius L. to toxic solutions. Jour. Exp. Biol. 24: 110. (Cited in U.S. EPA, 1980h.)

Lant, S.G. 1979. Diamond Shamrock. Personal communications to D. Goodwin, Office of Air Quality Planning and Standards. April 3, 1978. (Cited in Wagner et al., 1980).

Lillian, D., J.B. Singh, A. Appleby, L. Lobban, R. Aruts, R. Gompert, R. Hague, J. Tommey, J. Kazazis, M. Antell, D. Hansen and B. Scott. 1975a. Atmospheric fates of halogenated hydrocarbons. Environ. Sci. Technol. 9: 1042-1048.

Lillian, D., H.B. Singh, A. Appleby, L. Lobban, R. Arnts, R. Gompert, R. Hague, J. Toomey, J. Kazazis, M. Antell, D. Hansen and B. Scott. 1975b. Fates and levels of ambient halocarbons. Amer. Chem. Soc. Symp. Series. 17: 152-158.

Lowenbach and Schlesinger Associates. 1979. Chloroform: A Preliminary Materials Balance. Prepared for the Office of Toxic Substances, U.S. Environmental Protection Agency. Washington, DC. (Cited in Rem et al., 1982).

Mabey, W. and T. Mill. 1978. Critical review of hydrolysis of organic compounds in water under environmental conditions. Jour. Phys. Chem. Ref. Data. 7: 383-415.

Merck Index: An Encyclopedia of Chemicals and Drugs. 1976. M. Windholz, ed. 9th ed. Rahway, New Jersey. Merck and Co., Inc. p. 272.

Metcalf and Eddy, Inc. 1972. Waste Water Engineering, Collection Treatment Disposal. McGraw-Hill Book Co, NY. (Cited in Wagner et al., 1980).

Murray, A.J. and J.P. Riley. 1973. Occurrence of some chlorinated aliphatic hydrocarbons in the environment. Nature. 242: 37-38.

NAS (National Academy of Sciences). 1978. Chloroform, carbon tetrachloride, and other halomethanes: An environmental assessment. National Research Council. Washington, D.C. 294 pp.

NCASI (National Council for Air and Stream Improvement). 1977. Analysis of Volatile Halogenated Organic Compounds in Bleached Pulp Mill Effluent. NCASI Technical Bulletin No. 298. New York, NY. (Cited in Rem et al., 1982).

NCI (National Cancer Institute). 1976. Report on Carcinogenesis Bioassay of Chloroform. Available from National Technical Information Service, Springfield, Virginia. (NTIS PB-264-018).

NIOSH (National Institute for Occupational Safety and Health). 1974. Criteria for a recommended standard - occupation exposure to chloroform. Publ. No. (NIOSH) 75: 114. Dept. Health, Education, and Welfare. Washington, D.C.

NIOSH (National Institute for Occupational Safety and Health). 1977. Criteria for a recommended stnadard - occupational exposure to waste anesthetic gases and vapors. Publ. No. (NIOSH) 77-140. Department of Health, Education, and Welfare, Washington, D.C.

Okuno, T., M. Tsuji and K. Shintani. 1974. On the chlorination of hydrocarbons in air. Jour. Japan. Soc. Air Pollution. 9: 211.

Pearson, C.R. and G. McConnell. 1975. Chlorinate C_1 and C_2 hydrocarbons in the marine environment. Proc. R. Soc. Land B. 189: 305-332.

Pellizzari, E.D. 1977. The measurement of carcinogenic vapors in ambient atmosphere. U.S. Environmental Protection Agency. EPA-600/7-77-055.

Pellizzari, E.D., M.D. Erickson and R.A. Zweidinger. 1979. Analytical protocols for making a preliminary assessment of halogenated organic compounds in man and environmental media. U.S. Environmental Protection Agency. EPA-560/13-79-010. Available from NTIS PB80-109168.

Rasmussen, R.A., D.E. Harsch, P.H. Sweany, J.P. Krasnec and D.R. Crown. 1977. Determination of atmospheric halocarbons by a temperature-programmed gas chromatographic freezeout concentration method. Jour. Air Pollut. Contr. Distr. 27: 579-581.

Rem, R.M., M.E. Anderson, S.A. Duletsky, D.C. Misenheimer and H.F. Rollius. (1982). Chloroform Materials Balance, Draft Report. EPA contract 68-02-3168, Task 69.

Sax, N.I. (ed.). 1981. Dangerous Properties of Industrial Materials Report. Van Nostrand Reinhold Co. New York, NY. March/April. p.46. (Cited in Rem et al., 1982).

Singh, H.B. 1977. Atmospheric halocarbons: Evidence in favor of reduced average hydroxyl radical concentration in the troposphere. Geophys. Res. Lett. 4: 101-104.

Singh, H.B., L.J. Salas, H. Shigeishi, A.J. Smith, E. Scribner and L.H. Cavanagh. 1979. Atmospheric distributions, sources and sinks of selected halocarbons, hydrocarbons, SF_6, and N_2O. U.S. Environmental Protection Agency. EPA-600/3-79-107.

Singh, H.B., L.J. Salas, A. Smith and H. Shigeishi. 1980. Atmospheric measurements of selected toxic organic chemicals-interim report- 1979. U.S. Environmental Protection Agency, EPA-600/3-80-072. Avail. NTIS PB80-198989.

Singh, H.B., L.J. Salas, A.J. Smith and H. Shigeishi. 1981. Measurements of some potentially hazardous organic chemicals in urban environments. Atmos. Environ. 15: 601-612.

SRI International. 1983. 1983 Directory of chemical producers: United States of America. Menlo Park, California. SRI International. p. 502, 503, 584.

Stevens, A.A., C.J. Slocum, D.R. Seeger and G.G. Robeck. 1976. Chlorination of organics drinking water. Jour. Amer. Water Works Assoc. 68: 615-620.

Symons, J.M., T.A. Bellar, J.K. Carswell, J. DeMarco, K.L. Kropp, G.G. Robeck, D.R. Seeger, C.J. Slocum, B.L. Smith and A.A. Stevens. 1975. National Organics Reconnaissance Survey for Halogenated Organics. J. Am. Water Works Assoc. p. 634-647.

TAPPI (Technical Association of the Pulp and Paper Industry). 1963. The Bleaching of Pulp. TAPPI Monograph No. 27. New York, NY. (Cited in Rem et al., 1982).

U.S. EPA. n.d. National Organic Monitoring Survey. Technical Support Division, Office of Water Supply. Washington, DC. (Cited in Rem et al., 1982).

U.S. EPA. 1976. Atmospheric Freons and Halogenated Compounds. EPA-600/3-78-108. Environmental Sciences Research Laboratory, Research Triangle Park, NC. (Cited in Rem et al., 1982).

U.S. EPA. 1978a. In-depth studies on health and environmental impacts of selected water pollutants. U.S. Environmental Protection Agency. Contract No. 68-01-4646. (Cited in U.S. EPA, 1980h.)

U.S. EPA. 1978b. Statement of basis and purpose for an amendment to the national-interim primary drinking water regulations on trihalomethanes. Office of Water Supply, Washinton, D.C. (Cited in U.S. EPA, 1980h.)

U.S. EPA. 1979a. Fate of Priority Pollutants in Publicly Owned Treatment Works. EPA-400/1-70-301. Office of Water Regulations and Standards, Washington, DC. (Cited in Rem et al., 1982).

U.S. EPA. 1979b. Compilation of Air Pollution Emissions Factors, Third Edition -Supplement 12. AP-42. Transportation and Marketing of Petroleum Liquids. Office of Air Quality Planning and Standards, Research Triangle Park, NC. pp. 4.4-1 to 4.4-13. (Cited in Rem et al., 1982).

U.S. EPA. 1980a. Organic Chemical Manufacturing Volume 8: Selected Processes. Report 5: Chloromethanes by Methane Chlorination Process. EPA-450/3-80-028c. Office of Air Quality Planning and Standards. Research Triangle Park, NC. (Cited in Rem et al., 1982).

U.S. EPA. 1980b. Organic Manufacturing Volume 8: Selected Processes. Report 6: Chloromethanes by Methanol Hydrochlorination and Methyl Chloride Chlorination Process. EPA-450/3-80-028c. Office of Air Quality Planning and Standards. Research Triangle Park, NC. (Cited in Rem et al., 1982).

U.S. EPA. 1980c. Synthetic Organic Chemical Manufacturing Industry - Background Information for Proposed Standards, VOC Fugitive Emissions. EPA-450/3-80-33a. Office of Air Quality Planning and Standards. Research Triangle Park, NC. (Cited in Rem et al., 1982).

U.S. EPA. 1980d. Organic Chemical Manufacturing Volume 8: Selected Processes. Report 1: Ethylene Dichloride. EPA-450/3-80-028c. Office of Air Quality Planning and Standards. Research Triangle Park, NC. pp. III-1 to III-9. (Cited in Rem et al., 1982).

U.S. EPA. 1980e. Development Document for Effluent Limitations Guidelines and Standards for the Steam Electric Point Source Category. EPA-440/1-80-029b. Office of Water Regulations and Standards. Washington, DC. (Cited in Rem et al., 1982).

U.S. EPA. 1980f. Development Document for Effluent Limitations Guidelines and Standards for the Pulp, Paper and Paperboard and Builders' Paper and Board Mills. EPA-440/1-80-025b. Office of Water Regulations and Standards. Washington, DC. (Cited in Rem et al., 1982).

U.S. EPA. 1980g. Organic Chemical Manufacturing Volume 8: Selected Processes. Report 3: Fluorocarbon (Abbreviated Report). EPA-450/3-80-028c. Office of Air Quality Planning and Standards, Research Triangle Park, NC. p. III-1 to III-6. (Cited in Rem et al., 1982).

U.S. EPA. 1980h. Ambient Water Quality Criteria for Chloroform. Available from: National Technical Information Service, Springfield, Virginia. (NTIS PB81-117442).

U.S. EPA. 1981a. The determination of halogenated chemical indicators of industrial contamination in water by the purge and trap method. Method 502.1. U.S. Environmental Protection Agency, EPA-600/4-81-059.

U.S. EPA. 1981b. Air Pollution Emission Factors, Third Edition Supplement 12. AP-42. Storage of Organic Liquids. Office of Air Quality Planning and Standards. Research Triangle Park, NC. (Cited in Rem et al., 1982).

U.S. EPA. 1981c. The 1980 Needs Survey. EPA-430/9-81-008. Office of Water, Washington, DC. (Cited in Rem et al., 1982).

U.S. EPA. 1982a. Projections of Total Lead Usage Under Alternative Lead Phasedown Programs. Washington, DC. (Cited in Rem et al., 1982).

U.S. EPA. 1982b. Lead Phasedown Regulations. 47 FR 49322. November 1. (Cited in Rem et al., 1982).

U.S. EPA. 1982c. Chloroform Position Document 2. Office of Pesticides and Toxic Substances. Washington, DC. pp. 1-3. (Cited in Rem et al., 1982).

USITC. 1982. Synthetic organic chemicals. United States production and sales, 1981. U.S. International Trade Commission, Washington, D.C. USITC Publication 1292. p. 245.

Utidjian, H.M.D. 1976. Jour. Occup. Med. 18: 253. (Cited in ACGIH, 1980.)

Wagner, K., H. Bryson, G. Hunt, A. Shcohet. 1980. Draft Report Level I Materials balance chloroform. U.S. Environmental Protection Agency Contract No. 68-01-5793.

4. DISPOSITION AND RELEVANT PHARMACOKINETICS

4.1. INTRODUCTION

Considering that chloroform was the major anesthetic agent in use during the hundred years from its introduction by Simpson in 1847 (Waters, 1951; Snow, 1858; Simpson, 1847) until after the Second World War, there is relatively little detailed information about its pharmacokinetics and metabolism in man. This undoubtedly is due to the fact that until recently, specific and sensitive analytical methods were unavailable for the measurement of $CHCl_3$ and its metabolites at the concentrations in which they were likely to be present in vivo. Although chloroform as an anesthetic agent has been replaced by drugs with less cardiac and hepatic toxicity, it is still widely used in large bulk as an industrial solvent, as a chemical intermediary, and as a grain fumigant. Chloroform is present in the water supplies of many United States cities in concentrations reaching 311 μg/ℓ, and also has been indentified as a contaminant of the air (U.S. Occupational Safety and Health Administration (OSHA), 1978; National Institute for Occupational Safety and Health (NIOSH), 1977b; Dowty et al., 1975; Symons et al., 1975). Ordinary exposure to chloroform, therefore, includes occupational, food, drinking water, and ambient air (NIOSH, 1977b; Dowty et al., 1975; McConnell et al., 1975), hence, exposure can be chronic by both oral and pulmonary routes, but at levels far below anesthetic concentrations (5000 to 10,000 ppm; 24.85 to 49.70 g/m^3). Nonetheless, chloroform has been detected in the breath of healthy people living in non-industrial environments (Conkle et al., 1975) and in post-mortem human tissue samples (McConnell et al., 1975).

4.2. ABSORPTION

Chloroform is rapidly and extensively absorbed through the lungs and from the gastrointestinal tract. Inhalation is considered the primary route of entrance into man for occupational exposure and air pollution. Absorption after oral ingestion is of particular interest for chloroform as a contaminating component of drinking water and foodstuffs. Significant absorption through intact skin occurs only with liquid contact or submersion.

4.2.1. Dermal Absorption. Absorption of chloroform through the skin from direct liquid contact (immersion of hands or arms) is a slow process. Early studies (Torkelson et al., 1976; Schwenkenbecher, 1904; Witte, 1874) showed that chloroform does penetrate the skin and can be absorbed into the body by this route. Tsurata (1975, 1977) has studied the percutaneous absorption of a series of chlorinated organic solvents applied to a standard area of shaved abdominal mouse skin for 15 minute periods. Absorption was quantitated by presence of compound in total mouse body plus expired air, as determined by GC. For all solvents, percutaneous absorption linearly increased with time over the short exposure period and was directly related to water solubility. For chloroform the absorption rate was 329 moles/min/cm^2 skin, third highest of 8 solvents measured. This investigator extrapolated this absorption rate to a calculation of the amount absorbed into the human body as the result of 1 min immersion of both hands (800 cm^2 area). The estimated amount absorbed, 19.7 mg/min, was equated to an inhalation exposure concentration of 2429 ppm for 1 min. Tsurata concluded that skin absorption from liquid contact could be a significant route of entry into the body for chloroform. More recently Jakobson et al. (1983) carried out similar experiments with guinea pigs for 10 chlorinated organic solvents (which however did not include chloroform). Liquid contact (skin area, 3.1 cm^2) was maintained for up to 12 hours and solvent concentration in blood was monitored during, and for some solvents after exposure. For these solvents, the blood elimination curves following dermal exposure were non-linear, corresponding to a kinetic model involving at least two body compartments. Furthermore percutaneous absorption of these solvents, as reflected by blood concentration profiles, showed three different patterns that were related to water solubility. For solvents which were relatively hydrophilic [300 to 900 mg/100 ml water] the blood concentration increased steadily during the entire dermal exposure, indicating that absorption occurs faster than elimination by metabolism or pulmonary excretion. Chloroform with a water solubility of about 750 mg/100 ml water might be expected to be in this group.

4.2.2. Oral. The kinetics of gastrointestinal absorption of chloroform after oral ingestion have not been specifically studied; however, transmucosal diffusive passage occurs readily, as expected from its neutral and lipophilic properties (Tables 4-1 and 4-2), and as demonstrated by its biological effects produced by peroral administration of a wide range of dosages and dosing schedules for toxicity studies in rats, mice, guinea pigs, and dogs (Fishbein, 1979; Hill et al., 1975; Brown et al., 1974; Kimura et al., 1971; Klaassen and Plaa, 1967; Miklashevskii et al., 1966; Plaa et al., 1958; Eschenbrenner and Miller, 1945), teratology studies in rats and rabbits (Thompson et al., 1974), and metabolism studies in mice, rats, rabbits, monkeys, and man (Brown et al., 1974; Taylor et al., 1974; Fry et al., 1972; Rubinstein and Kanics, 1964; Paul and Rubinstein, 1963). Accidental and intentional ingestion of chloroform with rapid appearance of clinical symptoms also has been reported in man (Storms, 1973; Schroeder, 1965; Piersol et al., 1933).

TABLE 4-1

Physical Properties of Chloroform and Other Chloromethanes*

	Vapor Pressure at 25°C, torr	Ostwald Solubility, 37°C		
		Water/ air	Blood/ air	Olive Oil/ air
Dichloromethane	400	7.6	9.7	152
Chloroform	250	4.0	10.3	401
Carbon tetrachloride	100	0.25	2.4	361

*Source: Sato and Nakajima, 1979

Conversion factors:

20°C; 750 mmHg	1 ppm in air = 4.97 μg/ℓ = 4.97 mg/m^3
37°C; 760 mmHg	1 ppm in air = 4.69 μg/ℓ = 4.69 mg/m^3

TABLE 4-2

Partition Coefficients for Human Tissue at 37°C

Tissue	Coefficient	Relative to blood
Blood	8.0	
Brain		
Grey matter	16	2.0
White matter	24	3.0
Heart	8	1.0
Kidney	11	1.4
Liver	17	2.1
Lung	7	0.9
Muscle	12	1.5
Fat tissue	280	35.0

[a] Source: Steward et al., 1973

Brown et al. (1974) and Taylor et al. (1974) found that ^{14}C-chloroform in olive oil given perorally to mice, rats, and monkeys (60 mg/kg) was essentially completely absorbed by virtue of a 93 to 98% recovery of radioactivity in exhaled air, urine, and carcass (Table 4-9). Absorption was rapid, with peak blood levels at 1 hour in mice and monkeys. In man, Fry et al. (1972) observed that ^{13}C-chloroform (0.5 g) in olive oil swallowed in a gelatin capsule resulted in rapid appearance of the stable isotope in exhaled breath (Table 4-8), with peak blood levels at 1 hour.

Withey et al. (1982) have investigated the effect of dosing vehicle on the intestinal absorption of chloroform in fasting rats (400 gm) following intragastric intubation of equivalent doses (75 mg/kg) in about 4 ml of water or corn oil. The postabsorptive peak blood concentration averaged 6.5 times higher for water than corn oil (39 vs 6 μg/ml), while the time to initial peak blood concentration was essentially the same (5.6 vs. 6.0 min). Although the absorption from water vehicle exhibited one blood concentration peak, the absorption from corn oil showed two peaks in blood concentration at 6 and 40 minutes. The ratio of the areas under the blood concentration curves for 5 hours after dosing (AUC, 5 hr) was 8.7; water, corn oil. These results suggest that the absorption of chloroform with both vehicles is rapid; but, the rate and extent of absorption may be diminished, and the pattern of absorption altered by intragastric intubation of high volumes (for a rat) of corn oil vehicle. A slower partitioning of lipophilic compounds dissolved in corn oil with mucosal lipids can be expected in comparison with a water vehicle. Furthermore, in contrast to aqueous absorption into the portal system and thence to the liver, corn oil and other liquids are extensively transported via mucosal lymphatic system which slowly drains by way of the left lymphatic thoracic duct into the systemic circulation via the superior vena cava. While these considerations are unlikely to affect the pharmacokinetics of chloroform in man in any practical way, they are of importance in relation to the modes of dosing employed in long-term carcinogenicity tests of chloroform and other lipophilic compounds.

4.2.3. Pulmonary Absorption. Chloroform has a relatively high vapor pressure (250 torr at 25°C; Table 4-1) and a high blood/air partition coefficient (8 to 10.3 at 37°C; Table 4-2), and hence, its vapor in ambient air is a primary mode of exposure and the lungs a principal route of entry into the body. The total amount absorbed via the lungs (as for all vapors) is directly proportional to: (1) the concentration of the inspired air, (2) the duration in time of exposure, (3) the blood/air Ostwald solubility coefficient, (4) the solubility in the various body tissues, and (5) physical activity, which increases pulmonary ventilation rate and cardiac output. Hence, the basic kinetic parameters of the pulmonary absorption of chloroform and its equilibration in the body are as valid for low concentrations expected in the ambient environment as for the high vapor concentrations associated with its use as an anesthetic (5000 to 10,000 ppm; 24.85 to 49.70 g/m^3) (Smith et al., 1973; Morris, 1951; Waters, 1951). These parameters have not been as well studied as they have for modern anesthetics like halothane (Fiserova-Bergerova and Holaday, 1979) or even for other common halogenated hydrocarbon solvents like trichloroethylene, methylene chloride, or methylchloroform.

The earliest attempt at controlled studies of pulmonary absorption of chloroform in man were conducted by Lehmann and Hasegawa (1910). These investigators calculated retention values for chloroform (% inspired air concentration of chloroform retained in the body) from differences between inspired and expired air concentrations (analyzed by alkali hydrolysis with chloride titration). As expected, initial retention values were high, and decreased with exposure duration as total body equilibrium with inspired air concentration was approached (Table 4-3). The rate of uptake to equilibration and the final retention value achieved is related to the solubility of chloroform in blood (blood/air partition coefficient). Figure 4-1 illustrates for chloroform and other vapors that the greater the Ostwald solubility coefficient for a vapor agent, the less rapid equilibrium occurs. From the data of Lehmann and Hasegawa (Table 4-3) and recent data of Smith et al. (1973) of blood levels during anesthesia shown in Figure 4-2, total body equilibrium with inspired chloroform concentration requires at least ≥ 2 hours in normal man at resting ventilation rate and cardiac output. The retention value at equilibrium suggested by the Lehmann and Hasegawa (1910) data is ≈65%, and is 67% as calculated from the data of Smith et al. (1973). The difference, 33 to 36%, represents body elimination of chloroform by routes other than pulmonary (primarily by metabolism). The percent retention value is independent of the inspired air concentration at equilibrium.

The magnitude of chloroform pulmonary uptake into the body (dose, body burden) is directly related to the concentration of chloroform in the inspired air and to the duration of exposure. The total amount retained in the body during inhalation exposure can be estimated by multiplying percent retention (R) by the volume of air inspired during the exposure period, or:

$$\text{Amount uptake} = (C_I - C_A) \cdot V \cdot T$$

where V is ventilation rate (ℓ/minute), T is exposure period (minute), and C_I and C_A are inspired air concentration and end alveolar air concentration, respectively. Physical activity increases uptake by increasing the ventilation rate, V, and the cardiac output which influences rate of distribution to the various tissues of the body.

TABLE 4-3

Retention and Excretion of Chloroform in Man During and After Inhalation Exposure to Anesthetic Concentrations*

Subject	1	2	3
Inspired air conc. (ppm)	4448	4920	4407
	Retention (%)		
Exposure period (min)			
0 to 5	74.5	68.4	80.0
5 to 10	72.4	61.6	74.2
10 to 15	68.6	51.2	76.9
15 to 20	67.6	50.2	74.6
20 to 25	NR	NR	74.2
25 to 30	NR	NR	73.8
	Excretion, mg/ℓ expired air		
Postexposure (min)			
0 to 10	NR	NR	1.70
10 to 20	NR	NR	0.97
20 to 30	NR	NR	0.85

*Source: Lehmann and Hasegawa, 1910

NR = Not reported; min = minutes

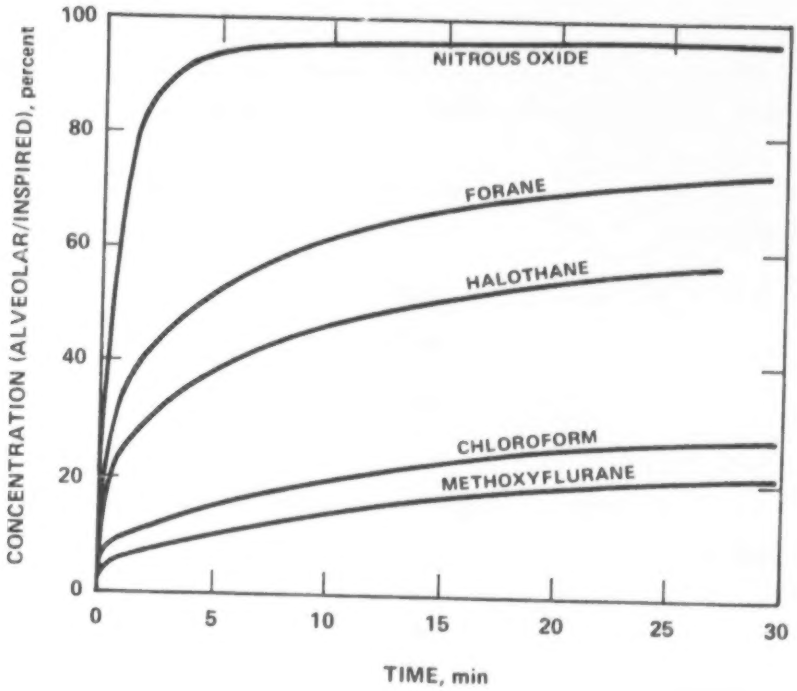

Figure 4-1. Rate of rise of alveolar (arterial) concentration toward inspired concentration for five anesthetic agents of differing Ostwald solubilities (blood/air partition coefficients): nitrous oxide, 0.47; forane, 1.4; halothane, 2.4; chloroform, 8; and methoxyflurane, 11. Note rate of alveolar chloroform rise is less than that of halothane with a smaller Ostwald coefficient and greater than that of methoxyflurane with a larger coefficient.

Source: Munson (1973)

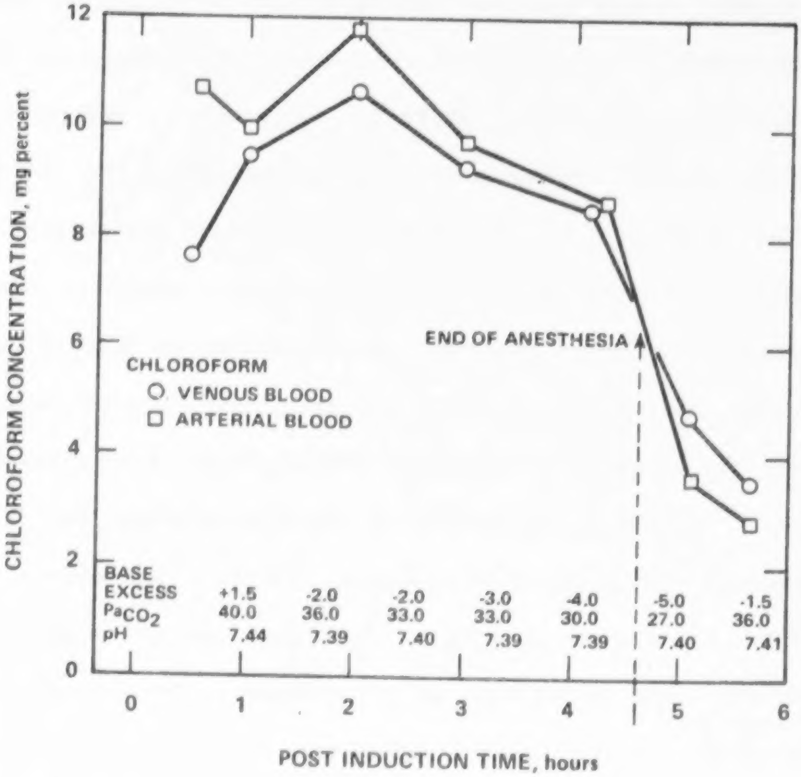

Figure 4-2. Arteriovenous blood concentrations of a patient during anesthesia with chloroform. Note anesthetic blood concentration for chloroform, the decreasing difference between arterial and venous concentrations at 2 to 3 hours, indicating whole-body equilibrium, and the rapid fall of blood concentration with termination of chloroform exposure.

Source: Smith et al. (1973).

During inhalation of chloroform (and in the post exposure elimination phase), the arterial blood concentration of chloroform is directly proportional to inspired air concentration (and end alveolar air concentration). This fixed relationship is defined by the blood/air partition coefficient in comparison to other solvents (Sato and Nakajima, 1979) (Table 4-1), and hence, for equivalent ambient air exposure concentrations, the blood concentration of chloroform is proportionally higher. For inspired air concentration required for surgical anesthesia (8,000 to 10,000 ppm; 39/76 to 49.70 g/m^3), Smith et al. (1973) observed a mean arterial blood chloroform concentration for 10 patients of 9.8 mg/dℓ with a range of 7 to 16.5 mg/dℓ (Figure 4-2), and Morris (1951) found similar values for his patients. For inspired air concentrations less than anesthetic levels, for example low vapor concentrations of 10 to 100 ppm (49.7 to 497 mg/m^3), blood chloroform concentrations are lower in direct proportion.

The amount of pulmonary absorption of chloroform is also influenced by total body weight and by the total fat content of the body (average body fat content is 8% of body weight) (Geigy Scientific Tables, 1973). The capacity of adipose tissue to absorb chloroform _in vivo_ is determined by the product of adipose tissue weight and lipid solubility of chloroform. The lipid solubility of chloroform is relatively high for this haloalkane (olive oil/air, 401; Tables 4-1 and 4-2), and also, the adipose tissue/blood partition coefficient is high (280 at 37°C); therefore, the uptake and storage of chloroform in adipose tissue can be substantial, and it is increased with excess body weight and obesity.

4.3. TISSUE DISTRIBUTION

Chloroform, after pulmonary or peroral absorption, is distributed into all body tissues. The compound crosses the placental barrier, as indicated by embryotoxicity and teratogenicity in mice, rats, and rabbits after oral and inhalation dosing (Murray et al., 1979; Dilley et al., 1977; Schwetz et al., 1974; Thompson et al., 1974). It has been found in fetal liver (von Oettingen, 1964). Chloroform can be expected to also appear in human colostrum and mature breast milk, since it has been found in fresh cow's milk and in high content in cheese and butter (Table 4-4).

As to be expected from its lipophilic nature and modest water solubility (Table 4-1), highest concentrations are found in tissues with higher lipid content; relative tissue concentrations are reflected by individual tissue/blood partition coefficients. Coefficients for human tissues, given in Table 4-2, indicate that relative tissue concentrations are expected in the order of adipose tissue > brain > liver > kidney > blood. The absolute amounts of chloroform found in these tissues at any given time are proportional to the body dose (i.e., to the concentration in the inspired air and duration of inhalation or to the oral dose, partition coefficient, and to the tissue compartment size).

Gettler (1934) and Gettler and Blume (1931), using a modified Fujiwara analytical method, determined the chloroform content of the brain, lungs, and liver of nine patients who died during surgical anesthesia (presumably 5000 to 10,000 ppm; 24.85 to 49.50 g/m^3 inspired air) as: brain, 120 to 182; lung, 92 to 145; and liver, 65 to 88 mg/kg tissue wet weight. Even higher values (372 to 480 mg/kg in brain tissue) were found in seven cases of death due to excessive administration of chloroform (Gettler, 1934). The blood concentration during surgical anesthesia has been recently determined (by GC) to range from 70 to 165 mg/ℓ in 10 patients (average, 98) by Smith et al. (1973). These tissue concentrations are in general agreement with the tissue/blood partition coefficients summarized from the literature by Steward et al. (1973) and given in Table 4-2.

TABLE 4-4

Chloroform Content in United Kingdom Foodstuffs and in Human Autopsy Tissue*

Chloroform in U.K. Foodstuffs		Chloroform in Human Autopsy Tissue			
Foodstuff	Chloroform µg/kg	Age of Subject	Sex	Tissue	Chloroform µg/kg (Wet tissue)
Dairy produce					
Fresh milk	5	76	F	Body fat	19
Cheshire cheese	33			Kidney	2
English butter	22			Liver	5
Hens eggs	1.4			Brain	4
Meat					
English beef (steak)	4	76	F	Body fat	5
English beef (fat)	3			Kidney	5
Pig's liver	1			Liver	1
				Brain	2
Oils and Fats					
Margarine	3	82	F	Body fat	67
Olive oil (Spanish)	10			Liver	8.7
Cod liver oil	6	48	M	Body fat	67
Vegetable cooking oil	2			Liver	9.5
Beverages					
Canned fruit drink	2	65	M	Body fat	64
Light ale	0.4			Liver	8.8
Canned orange juice	9	75	M	Body fat	65
Instant coffee	2			Liver	10.0
Tea (packet)	18				
		66	M	Body fat	68
Fruit and vegetables					
Potatoes (S. Wales)	18	74	F	Body fat	52
Potates (N.W. England)	4				
Apples	5				
Pears	2				
Tomatoes	2				
Fresh bread	2				

*Source: McConnell et al., 1975

In contrast to the high tissue levels of chloroform found in response to inspired air concentrations required for anesthesia, McConnell et al. (1975) recently analyzed post-mortem tissue from eight persons, four males and four females, with an age range of 48 to 82, living in the United Kingdom in ordinary non-industrial circumstances, for chloroform and other halogenated compounds (carbon tetrachloride, trichloroethylene, perchloroethylene, hexachlorobutadiene). Significant tissue levels of three chlorinated hydrocarbons were found. Chloroform level, µg/kg wet tissue weight, were: body fat, 5 to 68 (average of 51); liver, 1 to 10 (average of 7.2); kidney, 2 to 5; and brain, 2 to 4 (Table 4-4). Presumably, these tissue levels of chloroform were derived from air, foodstuff (Table 4-4), and drinking water contamination (OSHA, 1978; Dowty et al., 1975; Symons et al., 1975).

There have been few controlled exposure studies in animals investigating the distribution of chloroform in body tissues and determining dose-dependent tissue concentrations. Chenoweth et al. (1962) determined blood and tissue concentrations of chloroform in two normal fasted dogs after 2.5 hours of surgical anesthesia. Concentration of chloroform in the inhaled stream during anesthesia was not determined, but anesthesia was judged to be satisfactory at an arterial level of 45 to 50 mg/dℓ. Blood and tissue chloroform was determined by infrared spectroscopy after tissue extraction in cold carbon disulfide and distillation. Table 4-5 shows the relative concentration of chloroform in body tissues. The highest concentrations were found in fat tissue, some 10-fold greater than blood, and in adrenals (4-fold greater than blood); the concentrations in brain, liver, and kidney were similar to blood.

Cohen and Hood (1969) used low-temperature whole-body autoradiography to study the distribution of ^{14}C-chloroform in mice. Individual mice were administered 2.4 µℓ of ^{14}C-labeled chloroform by inhalation over a 10-minute

TABLE 4-5

Concentration of Chloroform in Various Tissues of Two Dogs After 2.5 Hours Anesthesia[a]

	Dog A	Dog B
	µg/gm wet tissue weight ± 5%[b]	
Arterial blood	275	397
Brain	298	392
Adrenal (total)	1185	1305
Fat, omentum	2820	1450
Right ventricle	214	314
Skeletal muscle	189	155
Lung	147	336
Liver	282	290
Spleen	237	255
Kidney	225	226
Bile	209	205
Thyroid	460	760
Pancreas	296	350
Urine	57	73

[a] Source: Chenoweth et al., 1962

[b] Chloroform concentration was determined by infrared spectrometry after tissue extraction.

period. The animals were sacrificed 0, 15, or 120 minutes after exposure. Autoradiography of mice killed immediately after inhalation showed the highest concentration of radioactivity in body fat and liver, while lesser and relatively uniform amounts were seen in blood, brain, lung, kidney, and muscle. By 120 minutes after exposure, a considerable decrease in total radioactivity occurred, now principally confined to liver, duodenum, and fat. A mottled appearance in the liver suggested a segmental or localized distribution. Biopsy specimens were taken from selected tissues in each animal and radioactivity determined by scintillation counting. Table 4-6 shows the distribution of radioactivity (chloroform and metabolites) in these tissues, and tissue/blood concentration ratios. Following sacrifice, after 10 minutes of exposure, most tissues approach a unit concentration with blood. However, in both fat and liver, the concentration exceeds unity. By 15 minutes, the ratio of radioactivity in brown fat reaches its peak at 15 times that found in blood. The relative concentration of radioactivity in the liver continues to increase until the termination of the experiment at 120 minutes, when it reaches a final value 6.7 times in excess of that in the blood. Kidney and lung tissues also increased in relative concentration over the 2-hour period to a value of 1.53 and 1.43 of blood, respectively. The increasing ratios of liver and kidney/blood radioactivity represent a continued accumulation of metabolites within these organs. High body fat/blood concentrations shows that adipose tissue represents an important storage site, prolonging retention of chloroform in the body.

Whole-body autoradiography was also carried out by Brown et al. (1974) on male and female Sprague-Dawley rats and squirrel monkeys given ^{14}C-chloroform perorally (60 mg/kg). Male and female rats killed 3 hours after dosage showed no apparent sex difference in distribution of radioactivity. Radioactivity was greatest in body fat and liver, while lesser amounts were seen in blood, brain,

TABLE 4-6

Concentrations of Radioactivity (Chloroform Plus Metabolites) in Various Tissues of the Mouse (NMRI)[a,b]

Tissue	Total Radioactivity (counts/min/mg)			Tissue/Blood Ratio		
	0 min	15 min	120 min	0 min	15 min	120 min
Blood	260 ± 22.0	103 ± 17.5	37 ± 4.0	1.00	1.00	1.00
Brain	217 ± 16.4	112 ± 9.9	23 ± 2.7	0.84	1.12	0.63
Muscle	288 ± 44.4	110 ± 5.1	26 ± 6.9	0.87	1.07	0.70
Lung	262 ± 24.1	149 ± 12.6	53 ± 8.2	1.01	1.44	1.43
Kidney	284 ± 35.0	145 ± 21.6	56 ± 8.0	1.08	1.41	1.53
Liver	407 ± 36.9	208 ± 9.3	250 ± 17.9	1.56	2.10	6.76
Fat	1674 ± 201	953 ± 92.7	266 ± 30.1	6.42	9.25	7.18
Brown fat	3158 ± 384	1490 ± 98.4	211 ± 40.3	12.12	14.70	5.70

[a] Source: Cohen and Hood, 1969

[b] Animal sacrifices were at 0, 15, or 120 minutes following 10-minute inhalation of chloroform. Data represent duplicate determinations in each of two animals at each time sequence (± S.E.)

Min = minutes

lung, kidney, and muscle. Squirrel monkeys showed a similar distribution, with the exception that high concentrations of radioactivity were present in the bile and increased with time. Examination of bile extract by gas-liquid chromatography showed the bile radioactivity was unchanged chloroform, indicating an excretion of chloroform by the biliary route in the monkey.

Brown and his colleagues (Taylor et al., 1974) also investigated the tissue distribution of chloroform in 3 strains of mice (CF/LP, CBA, and C57) by whole-body autoradiography after oral dosing (60 mg/kg ^{14}C-chloroform). In the male mice of the three strains examined 3 hours after dosing, the greatest amounts of radioactivity appeared in liver and kidneys, and lesser amounts in renal cortex but not medulla. Female mice showed greatest radioactivity in liver, intestine, and bladder, with much less radioactivity in kidney and little differentiation between renal cortex and medulla. The same general patterns were observed 5, 7, and 24 hours after dosing. Biopsy samples of these tissues were taken, and radioactivity determined by scintillation counting. Table 4-7 shows the distribution of ^{14}C-chloroform radioactivity in male and female mice killed 5 hours after dosing. There is a 3.5-fold difference between the activity present in the male and female kidneys of each strain. Male mice had greater activity in the kidneys, but female mice showed relatively greater activity in the liver. This sex difference in distribution was abolished by castration or testosterone administration to female mice. The sex difference in tissue distribution of chloroform and its metabolites may relate to the nephrotoxic effect of chloroform that occurs in male mice but not in female mice (Bennet and Whigham, 1964; Culliford and Hewitt, 1957; Hewitt, 1956; Shubik and Ritchie, 1953; Eschenbrenner and Miller, 1945a, 1945b). The pattern of tissue distribution of chloroform in mice also depends on mode of exposure. Cohen and Hood (1969), after chloroform inhalation, found highest levels in body fat (Table 4-6), while

TABLE 4-7

Tissue Distribution of ^{14}C-Chloroform Radioactivity in CF/LP Mice After Oral Administration (60 mg/kg)[a,b]

Tissue	Mean DPM/100 mg wet weight (SEM)	
	Male (6)	Female (6)
Liver	18,157 (1898)	21,535 (2097)
Kidney	13,759 (1047)	3920 (533)
Brown fat	1011 (80)	1074 (54)
Blood	2910 (423)	2906 (457)

[a] Source: Taylor et al., 1974

[b] Similar results were obtained for CBA and C57 strains.

Brown et al. (1974) and Taylor et al. (1974) observed lower levels in fat and highest levels in liver and kidney following oral dosing (Table 4-7). The high liver levels of chloroform after oral administration may be due, in part, to first passage and extraction by the liver after this route of administration, to differences of time after exposure (2 versus 5 hours), and to metabolism and covalent binding of metabolites to cellular macromolecules (see below).

It is worth re-emphasizing that the sex difference in tissue distribution and binding of chloroform (and metabolites) in kidney and liver, noted by Brown and his colleagues (Taylor et al., 1974), appeared to be peculiar to mice and that these workers did not observe such differences in male and female rats or squirrel monkeys (Brown et al., 1974).

4.4. EXCRETION

Elimination of chloroform from the body is perforce the sum of metabolism and excretion of unchanged chloroform via pulmonary and other routes. Unmetabolized chloroform is excreted almost exclusively through the lungs; however, metabolism of chloroform is extensive, with the proportion excreted unchanged dependent on body dose. Surprisingly, considering its historical importance, its longtime use as an industrial chemical and anesthetic agent, few controlled experimental studies in man have been made on the kinetics of excretion of chloroform.

4.4.1. Pulmonary Excretion. Figure 4-3 shows the time-course of pulmonary elimination of chloroform after accidental inhalation exposure to a mixture of solvents, including chloroform, carbon tetrachloride, trichloroethylene, and perchloroethylene. Stewart et al. (1965) determined, post-exposure, the alveolar

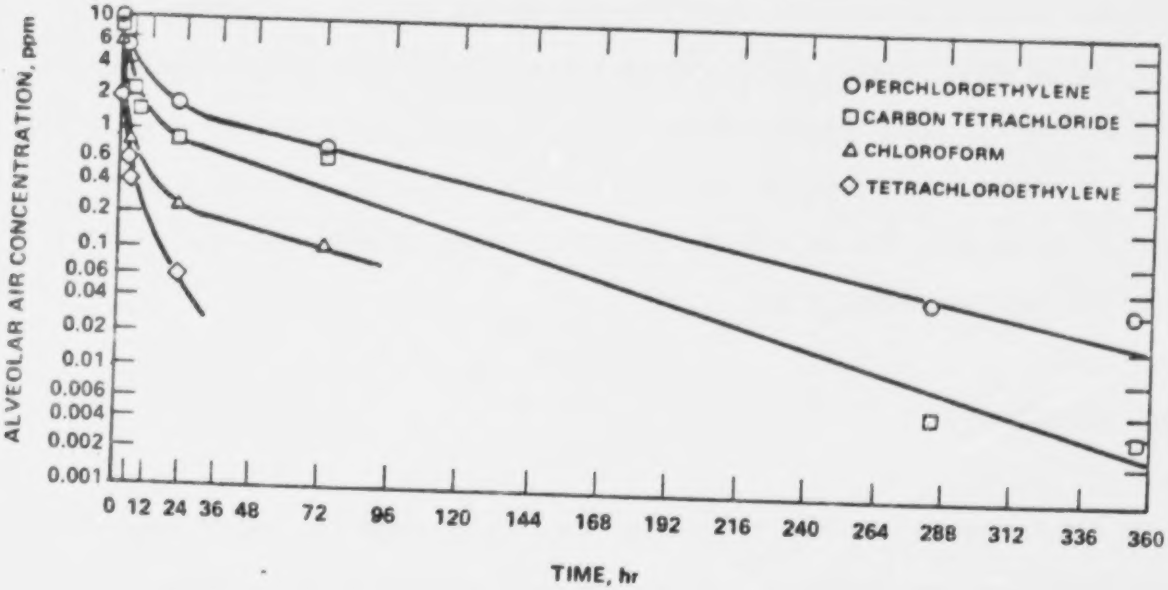

Figure 4-3. Exponential decay of chloroform, carbon tetrachloride, perchloroethylene and trichloroethylene in exhaled breath of 48 year-old male accidentially exposed to vapors of these solvents. The alveolar air (end tidal) was collected in a 50-mℓ glass pipette, and the sample was analyzed by infrared spectroscopy and gas-liquid chromatography. Initial values (30 min. post exposure) were chloroform, 7 ppm; carbon tetrachloride, 9.5 ppm; perchloroethylene, 11 ppm; and trichloroethylene, 4 ppm.

Source: Stewart et al. (1965).

air concentrations of these solvents by infrared and gas-liquid chromatography analysis. The kinetics of pulmonary excretion of the solvents are independent of one another. However, all, including chloroform, demonstrate the typical kinetics of gaseous vapor pulmonary elimination, that has been observed experimentally for relatively hydrophobic, volatile gaseous anesthetics and industrial solvents (Eger, 1963; Fiserova-Bergerova et al., 1974, 1979, 1980; Droz et al., 1977). At termination of exposure with zero concentration in inspired air, chloroform (and the other solvents) immediately begins to be eliminated from the body into the lungs, with blood and alveolar air concentrations describing parallel exponential decay curves with three major components (Figure 4-3). These exponential components have been related by many investigators (Eger, 1963; Fiserova-Bergerova et al., 1974, 1979, 1980; Droz et al., 1977) to first-order kinetics of pulmonary elimination associated with desaturation of physiological compartments in accordance with a blood flow-limited model in which the rate constants are determined predominately by tissue perfusion, volume of tissue distribution and by partition coefficients:

$$\begin{array}{l}\text{Tissue uptake and}\\ \text{desaturation of}\\ \text{compartment,T}\end{array} = F_T \cdot \lambda_{bl/air} \exp\left(- \frac{F_T}{V_T \, \lambda_{T/bl}} \cdot t \right)$$

where F is blood flow through tissue compartment, V is volume of the compartment, λ is partition coefficient, and exp is base of natural logarithm.

$$t_{1/2} = \frac{0.693 \, V \, \lambda_{T/bl}}{F}$$

Since three exponential components are typically observed experimentally, three physiological tissue compartments are included in the model described by a three-term exponential function of the form:

$$\text{Uptake, Desaturation} = A\,e^{-\alpha t} + B\,e^{-\beta t} + C\,e^{-\gamma t}$$

where A, B, C are macrocoefficients and α, β, γ, are hybrid constants (defined above). These terms represent three flow-limited major body compartments (1) a vessel-rich group of tissues (VRG) with high blood flow and high diffusion rate constant (VRG: brain, heart, kidneys, liver and endocrine and digestive systems), (2) lean body mass (MG: muscle and skin), and (3) adipose tissue (FG). More recently, Fiserova-Bergerova and coworkers (1974, 1979, 1980) have mathematically re-formulated this physiological, first-order model to accomodate the effect of metabolism on uptake, distribution and clearance of inhaled vapor compounds.

The half-time (t 1/2) of elimination from the physiological compartments (VRG < MG < FG) are independent of the body dose, but are dependent on tissue/blood partition coefficients and blood/air partition coefficients. Since these solvent compounds have high solubility in body fat (Table 4-1), they are eliminated slowly from fat depots with long half-time of elimination, as illustrated by Stewart's patient (Stewart et al., 1965) in Figure 4-3. From Figure 4-3, it can be estimated graphically that chloroform has a half-time of elimination from the fat compartment (FG) of ≃36 hours, with similar long half-times for the highly fat soluble compounds, perchloroethylene and carbon tetrachloride.

There is little information available for the half-times of pulmonary elimination of chloroform from the VRG and MG. From the early data of Lehmann and Hasegawa (1910) given in Table 4-3, the half-time of pulmonary elimination from the VRG appears to be ≃30 minutes. A similar estimate can be made from the data of Smith et al. (1973) and Morris (1951) at termination of anesthesia in man; these workers found that blood chloroform concentration rapidly fell exponentially from 7 to 3.5 mg/dℓ within 30 minutes (Figure 4-2).

Pulmonary elimination of chloroform was investigated by Fry et al. (1972) in male and female volunteers given ^{13}C-chloroform in olive oil orally (by gelatin capsule). Chloroform was determined in expired air by GLC. Their data, summarized in Table 4-8, show that the amount of chloroform excreted through the lungs within 8 hours (expressed as a percentage of the dose, 0.1 to 1.0 g), increased (0 to 65%) in proportion to the dose. Following a peak blood concentration (0.5 mg/dℓ for a 500 mg dose) 1 hour after oral dosage, absorption, and distribution, the blood chloroform concentration declined exponentially with three components: (1) a very rapid disappearance, with half-time of 14 minutes possibly corresponding to VRG compartment kinetics, (2) a slower disappearance, with half-time of 90 minutes corresponding to MG kinetics, and (3) a very slow disappearance, with very long half-time from adipose tissue. This half-time was undetermined, but chloroform was detected in blood and breath 24 hours later. Fry and coworkers (1972) noted a linear relationship for their subjects between pulmonary excretion of chloroform and body weight deviation from ideal, an index of excessive leanness or excessive body fat from normal. Their data in Figure 4-4 show that for both male and female subjects given a standard oral dose of chloroform, lean subjects eliminate via the lungs a greater percentage of the dose, while overweight subjects eliminate less chloroform. The different slopes of the linear relationship for men and women presumably reflect the different proportion of adipose tissue in the two sexes. The bodies of women tend to contain higher proportions of fat than those of men (Geigy Scientific Tables, 1973). These observations reinforce the role of adipose tissue as a storage site for chloroform.

Brown and his coworkers (Brown et al., 1974; Taylor et al., 1974) have demonstrated an animal species difference in the amount of pulmonary excretion of ^{14}C-chloroform from a standard oral dose (60 mg/kg body weight) given in olive

TABLE 4-8

Pulmonary Excretion of $^{13}CHCl_3$ Following Oral Dose: Percent of Dose[a]

Subjects	Dose (g)	Mean for 8 Hours[b]	Range
8 M and F	0.5	40.3	17.8 to 66.6
1	1.0	64.7	NA
1	0.25	12.4	NA
1	0.10	nil	NA

Pulmonary excretion of $^{13}CO_2$ following 0.5 g oral dose of $^{13}CHCl_3$: Cumulative percent of dose[b]

	Time after dose (hours)				
Subjects	0.5	1.75	2.5	5.5	7.5
Male (1)	2.1	24.1	35.9	49.2	50.6
Female (62.7 kg) (1)	0.5	10.7	28.3	47.5	48.5

[a] Recalculated from the data of Fry et al., 1972

[b] Within 4% of value calculated for infinite time

NA = Not applicable

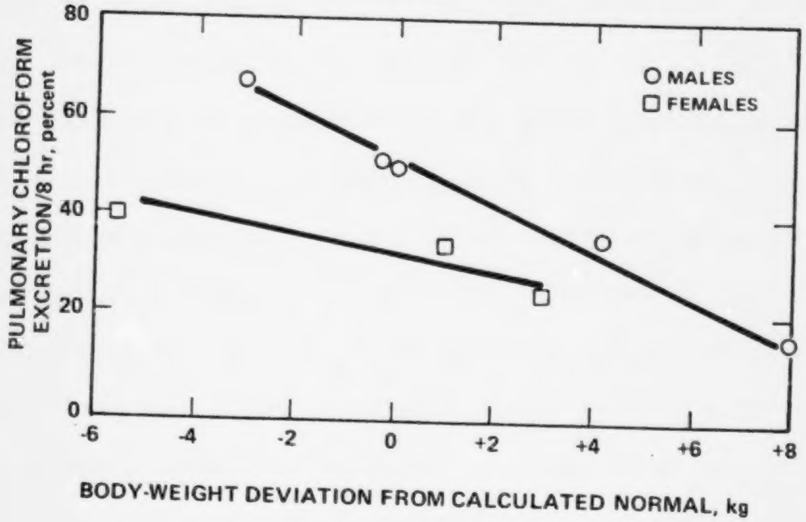

Figure 4-4. Relationship between total 8-hour pulmonary excretion of chloroform following 0.5-g oral dose in man and the deviation of body weight from ideal. The different slopes of the linear relationship for men and women reflect the different proportion of adipose tissue in the two sexes.

oil. Mice (three strains), rats, and squirrel monkeys excrete chloroform via the lungs (6, 20, and 79%, respectively, of the standard dose). This species difference is primarily related to the capacity to metabolize chloroform rather than differences in pulmonary kinetics, since, as shown in Table 4-9, the percentage of the dose metabolized to $^{14}CO_2$ is inversely proportional to that of pulmonary excretion. The mice, 48 hours after dosing, retained only 2% of chloroform radioactivity (Table 4-9).

Withey and Collins (1980) determined the kinetics of distribution and elimination of chloroform from blood of Wistar rats after intravenous administration of 3, 6, 9, 12 or 15 m/kg of chloroform given in 1 ml water intrajugularly. For all doses, the blood decay curves exhibited three components of exponential disappearance of chloroform (α, β, γ components) and "best" fitted a first-order three compartment model. Table 4-9b summarized the values obtained for the kinetic parameters. For volatile, lipophilic compounds, for which a major route of elimination is pulmonary, experiments utilizing dose administration via relatively large intravenous bolus injections (relative to rat total blood volume), and which measures only blood chloroform disappearance, provide a number of problems for data interpretation of elimination (pulmonary and metabolism) and/or tissue distribution. In these experiments, pulmonary elimination, which is rapid for organic solvents, occurs simultaneously with distribution and metabolism; in contrast, experiments in which the animal is preloaded by oral or inhalation administration, distribution is more readily separable from pulmonary elimination. After intravenous administration (Table 4-9b), the rate constant k_e (for elimination of chloroform from the central compartment blood out of the body (principally via pulmonary excretion and/or metabolism) was dose-dependent and consistent with a half-time of elimination of only 3.6 min for the lowest dose and only 6.2 min for the highest dose. Since the

TABLE 4-9

Species Difference in the Metabolism of ^{14}C-Chloroform (Oral Dose of 60 mg/kg)*

Species	No.	^{14}C-radioactivity 48 hours after dose Mean values as percent dose				
		Expired $^{14}CHCl_3$ or metabolites	Expired $^{14}CO_2$	Urine + Feces	Carcass	Total
Mice CF/LP, CBA, C57 strains	19	6.1	85.1	2.6	1.8	95.6
Rats S-D	6	19.7	65.9	7.6	NR	93.2
Squirrel Monkeys	6	78.7	17.6	2.0	NR	98.3

*Recalculated from the data of Brown et al., 1974

NR = Not recorded

Table 4-10. Kinetic Parameters for Chloroform After I.V. Administration to Rats

Dose* mg/kg	V_d ml	α	β	γ	k_e	k_{12}	k_{21}	k_{13}	k_{31}
					min^{-1} ± S.E.				
3.0	45.07 ± 0.04	0.72 ± 0.11	0.135 ± 0.001	0.0287 ± 0.0064	0.1907 ± 0.0239	0.2346 ± 0.0651	0.2575 ± 0.0316	0.0921 ± 0.0008	0.0421 ± 0.0098
6.0	53.57 ± 12.61	0.64 ± 0.13	0.081 ± 0.01	0.0158 ± 0.0019	0.1874 ± 0.0356	0.2681 ± 0.0631	0.1862 ± 0.0188	0.0730 ± 0.0246	0.0233 ± 0.0032
9.0	64.46 ± 14.26	0.64 ± 0.084	0.095 ± 0.0001	0.0189 ± 0.0009	0.1284 ± 0.0217	0.2529 ± 0.0489	0.2421 ± 0.0064	0.0594 ± 0.0034	0.0281 ± 0.0042
12.0	80.62 ± 20.20	0.32 ± 0.20	0.060 ± 0.028	0.0074 ± 0.0056	0.1035 ± 0.0232	0.1071 ± 0.0774	0.1192 ± 0.0676	0.0395 ± 0.0253	0.0106 ± 0.0084
15.0	89.13 ± 12.83	0.35 ± 0.048	0.070 ± 0.006	0.0134 ± 0.0005	0.1124 ± 0.0110	0.1104 ± 0.0256	0.1523 ± 0.0188	0.0396 ± 0.0104	0.0193 ± 0.0018
15.0; $t_{\frac{1}{2}}$ min		2.1	9.9	51.7	6.2	6.3	4.6	17.5	35.9

*2 to 4 rats/dose . From Withey et al., 1982.

half-times for distribution into other tissue compartments from the central compartment blood have longer half-times, it is likely that, of the dose introduced into the blood, a major portion (depending on dose) was excreted within a few minutes by the lungs. Further indication that the dose and/or mode of administration influenced the distribution and elimination of chloroform was shown by the proportional increase of the apparent volume of distribution, V_d (45 ml; 3 mg/kg to 89 ml; 15 mg/kg) and the decrease with dose in values for rate constants of transfer from blood to other tissue compartments. The volume of distribution (V_d) of chloroform was 89 ml for the highest dose or about 22% b.w., surprisingly low for a lipid soluble compound that is known to diffuse into all the major organ systems (Tables 4-5 and 4-6). Adipose tissue is known to be a major tissue compartment for chloroform [Section 4.3]; the clearance of chloroform from perirenal fat was found to be slow with a half-time of 106 min, and since the rate constant, k_{31} was given as 0.0193 min^{-1} (for 15 mg/kg dose) indicating a half-time of 36 min, the adipose tissue appears to be a deep compartment. These investigators believe that their kinetic data shows no evidence in the rat of nonlinear or dose-dependent Michaelis-Menten kinetics and they suggest that a dose of 15 mg/kg is below hepatic metabolism saturation.

4.4.2. Other Routes of Excretion. Chloroform is not eliminated in significant amounts from the body by any route other than pulmonary. Studies of chlorinated compounds in the urine after chloroform inhalation exposure or peroral dosage to animals and humans have failed to detect unchanged chloroform (Brown et al., 1974; Fry et al., 1972). Brown et al. (1974) identified chloroform in high concentration in the bile of squirrel monkeys after oral ^{14}C-chloroform dosage, and suggested an active enterohepatic circulation in this species. For monkeys, they found only 2% of dose radioactivity in combined urine and feces collected for 48 hours after dosing (Table 4-9), and only 8 and 3% for rats and mice, respectively.

4.4.3. Adipose Tissue Storage. There is no definitive experimental evidence in the literature concerning bioaccumulation after chronic or repeated daily exposure to chloroform. However, there are practical reasons to believe that extended residence in body fat occurs. In man, chloroform has a relatively high fat tissue/blood partition coefficient of 35 (Table 4-2), a long half-time of elimination from adipose tissue compartments of ≈36 hours (Figure 4-3), and it has been detected in blood and breath 24 to 72 hours after a single exposure (Stewart, 1974; Fry et al., 1972; Stewart et al., 1965.). Figure 4-5 clearly shows the slow elimination of chloroform from the adipose tissue of dogs following a 3-hour anesthesia (Chenoweth et al., 1962). Despite the rapid exponential decline of blood levels of chloroform within 3 hours after termination of anesthesia, significant levels of chloroform were still present 20 hours later.

Fry et al. (1972) has provided indirect evidence in man of the storage of chloroform in body fat (Figure 4-4), while analysis of body fat of animals given a single inhalation exposure or oral dosage demonstrated marked accumulation of chloroform in this tissue (Taylor et al., 1974; Cohen and Hood, 1969) (Tables 4-5, 4-6, 4-7). Particularly pertinent are the observations of McConnell et al. (1975), who demonstrated the occurrence of significant amounts of chloroform (and other chlorinated hydrocarbons) in autopsy tissues (highest concentrations in body fat) of humans exposed only to ordinary ambient air (Table 4-4), and of Conkle et al. (1975), who analyzed the GC-MS alveolar air of eight fasting healthy men working in a nonindustrial environment and found in three men

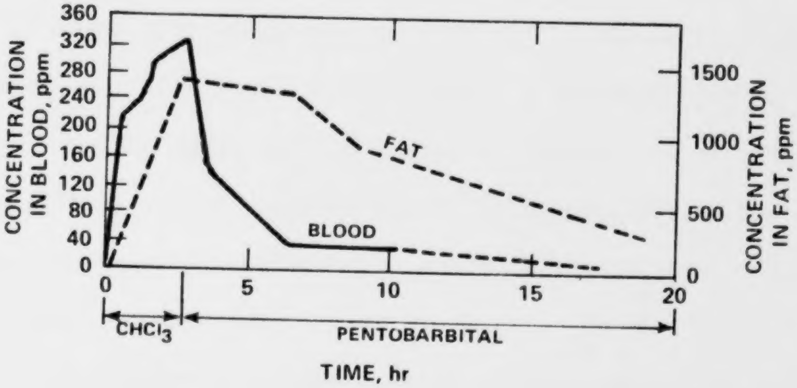

Figure 4-5. Blood and adipose tissue concentrations of chloroform during and after anesthesia in a dog. Note the high prolonged levels of chloroform in adipose tissue (broken line) for 20 hours even after rapid exponential fall in blood concentration (solid line) with termination of chloroform anesthesia after three hours.

Source: Chenoweth et al. (1962).

significant rates of pulmonary excretion of chloroform (Table 4-10), as well as other halocarbons (for example, methylene chloride, dichlorobenzene, methylchloroform) in all eight men.

4.5. BIOTRANSFORMATION OF CHLOROFORM

4.5.1. Known Metabolites. The haloforms, and chloroform in particular, long have been known to undergo extensive mammalian biotransformation. Zeller (1883) clearly demonstrated an increased daily urinary inorganic chloride excretion representing 25 to 60% of the dose in dogs given oral doses of chloroform (7 to 10 g in gelatin capsules). Eighty years later, Van Dyke et al. (1964), using ^{36}Cl-chloroform, confirmed in rats that the extra urinary inorganic chloride originated from the metabolism of chloroform. Zeller (1883) also found, in the urine of his dogs given chloroform, a levo-rotatory oxidative metabolite that he suggested to be the glucuronide of trichloromethanol, a compound only recently postulated as an intermediate of P_{450} oxidative metabolism (Figure 4-6). Van Dyke and coworkers (1964) also found evidence for ^{36}Cl-metabolites (≈2% dose) in the urine of their rats given chloroform. However, other investigators (Brown et al., 1974; Fry et al., 1972; Paul and Rubinstein, 1963; Butler, 1961) with newer methodologies have not been able to identify lesser chloromethanes in the urine or breath of mouse, rat, or man after chloroform exposure.

In addition to the chloride ion, it has been established from both in vivo and in vitro studies that the major end-product metabolite of chloroform is carbon dioxide (CO_2) (Brown et al., 1974; Fry et al., 1972; Rubinstein and Kanics, 1964; Van Dyke et al., 1964; Paul and Rubinstein, 1963), with phosgene identified as the immediate precursor metabolite from in vitro studies (Mansuy et al., 1977; Pohl et al., 1977; Ilett et al., 1973) (Figure 4-6). CO_2 from

TABLE 4-11

Levels of Chloroform in Breath of Fasted Normal Healthy Men*

Subject	Age	Chloroform excretion, μg/hr
A	34	2.0
B	28	ND
C	33	ND
D	38	ND
E	47	11.0
F	28	ND
G	38	ND
H	23	0.22

*Source: Conkle et al., 1975

ND = Not detected

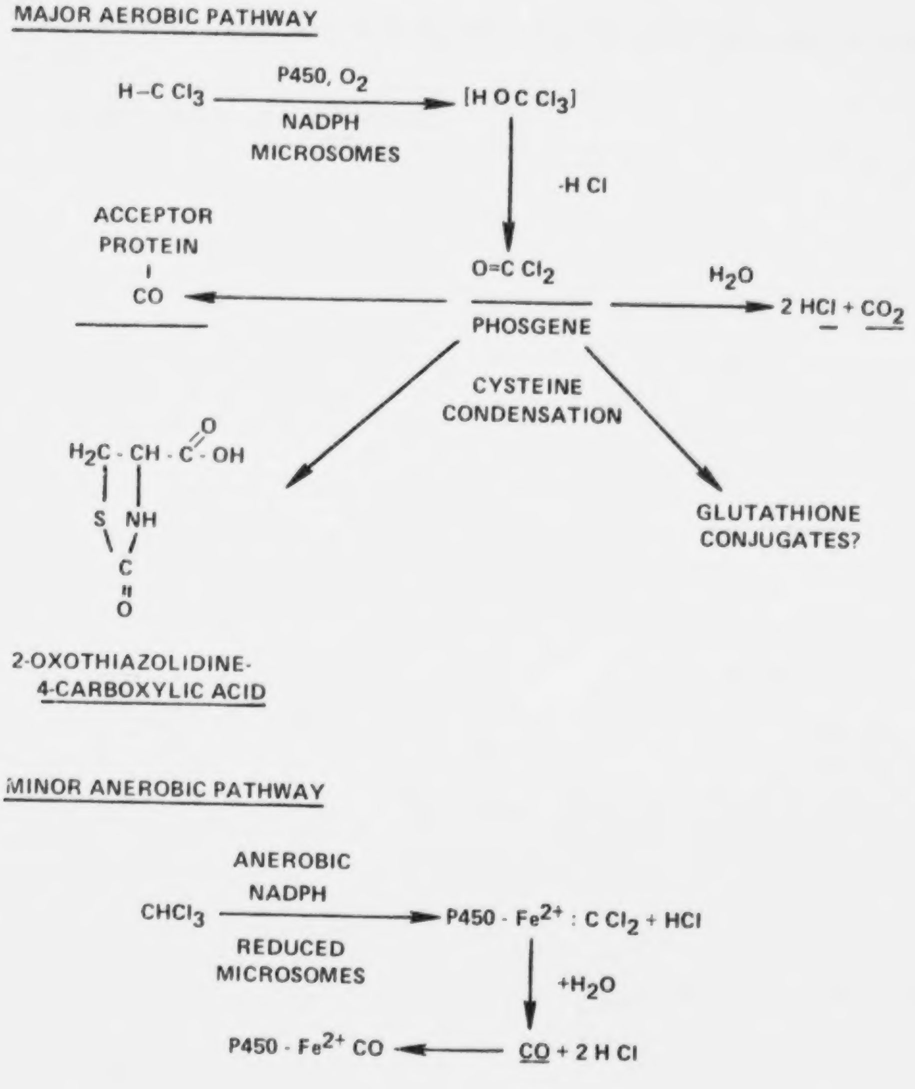

Figure 4-6. Metabolic pathways of chloroform biotransformation. (Identified CH Cl_3 metabolites are underlined.)

chloroform metabolism is primarily excreted through the lungs, but a small percentage (<7%) is incorporated into endogenous metabolites and excreted into the urine as bicarbonate, urea, methionine, and other amino acids (Brown et al., 1974). Carbon monoxide (CO) has also been identified as a very minor metabolite of anaerobic chloroform metabolism (Figure 4-6), both from in vitro studies (Ahmed et al., 1977; Wolf et al., 1977) and in vivo animal studies (Anders et al., 1978; Bellar et al., 1974).

In addition to chloroform metabolites that are excreted, phosgene and other "reactive intermediates" of chloroform metabolism interact with and covalently bind to tissue acceptors such as protein and lipids (Docks and Krisna, 1976; Uehleke and Werner, 1975; Brown et al., 1974; Ilett et al., 1973; Cohen and Hood, 1969; Reynolds, 1967; Cessi et al., 1966).

The liver is the principal site of chloroform metabolism, although Paul and Rubinstein (1963) and Butler (1961) found that rat kidney, adipose tissue, and skeletal muscle also converted chloroform to CO_2 (25, 0.8, and 8% of liver, respectively).

4.5.2. Magnitude of Chloroform Metabolism. Chloroform is metabolized to differing extents in man and other animal species. Since chloroform and other halogenated hydrocarbons are thought to produce pathological effects by metabolism in target tissues to reactive intermediates that covalently bind to macromolecules (Chapter 5), the total capacity to metabolize chloroform as well as individual tissue sites of metabolism are important determinants of expected interspecies differences in toxic susceptibility. This interdependence between intensity of toxic response and metabolism, and interspecies differences in magnitude of metabolism, are important considerations in extrapolation from experimental animal to man (Reitz et al., 1978).

Few studies have been made of the capacity of man to metabolize chloroform; virtually no studies have been made of the pharmacokinetic, endocrine, genetic, and environmental factors modifying metabolism in man. The early studies of Lehmann and Hasegawa (1910) on the retention of chloroform from inspired air inhaled by three volunteers (4500 to 5000 ppm, average of 64%) (Table 4-3) suggest that 36% of pulmonary uptake of chloroform in man is metabolized. Similarly, a retention value of 67%, calculated from the data of Smith et al. (1973) for patients inhaling 10,000 ppm chloroform during surgical anesthesia (Figure 4-2), indicates 33% chloroform metabolism during inhalation exposure. A similar estimate of the extent of chloroform metabolism during anesthesia has been made by Feingold and Holaday (1977). These workers simulated, with a computer, chloroform inhalation kinetics using a non-linear whole-body compartmental model, and found that the percent of chloroform uptake metabolized was 30%. This rate of metabolism remained constant during 8 hours of anesthesia, and continued for several days following termination of anesthesia, presumably from chloroform stored during anesthesia in adipose tissue.

Fry et al. (1972) have investigated the metabolism of chloroform in man after single oral doses. Isotopically labeled ^{13}C-chloroform dissolved in 1.0 mℓ olive oil/gelatin capsule was given to 12 healthy male and female volunteers (58 to 60 kg body weight) at doses of 0.1 to 1.0 g. For two of the volunteers, pulmonary excretion of $^{13}CO_2$ in expired air from the metabolism of chloroform was serially connected over a 7.5 hour period and analyzed by mass spectrometry. The results given in Table 4-8 show that 49 and 51% of a 0.5 g dose was metabolized to $^{13}CO_2$. Pulmonary excretion of unchanged ^{13}C-chloroform during a comparable period (8 hours) in a separate experiment with these two subjects were 67 and 40%, respectively. No metabolites other than CO_2 (e.g., methylene dichloride, tetrachloroethane) were found in expired air, and chloroform was not found in the urine. These results indicate that: (1) virtually all of an oral chloroform dose (0.5 g) can be accounted for by pulmonary excretion of CO_2 and unchanged chloroform, (2) metabolism of chloroform to CO_2 is ≈50% of this dose, and (3) absorption and metabolism are rapid and virtually complete within 5 hours as shown in Table 4-8, possibly because of first pass through the liver. In this respect, the kinetics of metabolism of oral doses may differ substantially from inhalation doses. Furthermore, the data of Table 4-8 suggest that the fraction of the dose metabolized is dose-dependent. Thus, an oral dose of 0.1 g was completely metabolized (100%), with no chloroform excreted unchanged through the lungs; but for a 1.0 g dose, 65% was excreted and only 35% metabolized. These results suggest that metabolism is rate-limited in man, since a diminishing proportion of dose is metabolized with increasing dose (Table 4-8).

In man, Chiou (1975) has shown that up to 38% of an oral dose of chloroform is metabolized in the liver, and up to about 17% is excreted intact from the lungs before the chloroform reaches the systemic circulation, an example of a first-pass effect.

Animal experiments demonstrate a marked species difference in the metabolism of chloroform. Early experiments in the 1960's by Paul and Rubinstein (1963), Van Dyke et al. (1964), and Cohen and Hood (1969) with mice and rats given ^{14}C-chloroform indicated a minimal metabolism of chloroform (≈4%) occurred in these species. More recent studies by Brown and his coworkers (Brown et al., 1974; Taylor et al., 1974) have shown that mice and rats metabolize chloroform to CO_2 extensively (65 to 85%), and to a greater extent than non-human primates or man. These investigators gave equivalent oral doses (60 mg/kg) of ^{14}C-chloroform to mice (3 strains), rats, and squirrel monkeys, and determined ^{14}C-labeled chloroform or volatile metabolites and $^{14}CO_2$ in expired air, ^{14}C-radioactivity in urine, and ^{14}C-radioactivity remaining in animals at sacrifice 4 to 8 hours after dosing. Total recovery of ^{14}C-chloroform radioactivity was excellent and accounted for 93 to 98% of the administered dose. Their results are summarized in Table 4-9. Using $^{14}CO_2$ as a measure of the fraction of the chloroform dose metabolized (intermediate chloromethane metabolites were not found in breath or urine), mice metabolized 85% of the dose, rats, 66%, and squirrel monkeys, 18%. A further 2 to 8% of ^{14}C-radioactivity ($^{14}CO_2$ incorporated into urea, bicarbonate, and amino acids) were found in urine. They found no strain difference in mice, or sex difference in mice, rats, or monkeys in capacity to metabolize chloroform, or in tissue distribution and binding of metabolites, except for mice, where kidney radioactivity concentration was greater in males than females and lesser in livers of males than females (Table 4-7). These findings of Brown and coworkers of large interspecies differences for metabolism of chloroform and marked sex differences in mice (but not other species) for tissue distribution and covalent binding of intermediate metabolites to tissue macromolecules in liver and kidney emphasize the difficulties and dangers of extrapolating studies in lower animals to man (Reitz et al., 1978).

4.5.3. Enzymic Pathways of Biotransformation. It has been postulated for many years (Docks and Krishna, 1976; Uehleke and Werner, 1975; Brown et al., 1974; Ilett et al., 1973; Reynolds, 1967; Paul and Rubinstein, 1963) that a reactive metabolite of $CHCl_3$ is responsible for its liver and renal toxicity in man (von Oettingen, 1964; Conlon, 1963) and experimental animals (Bhooshan et al., 1977; Pohl et al., 1977; Ilett et al., 1973; Klaassen and Plaa, 1966), and possibly the production of liver tumors in mice (Eschenbrenner and Miller, 1945a). For example, when rats or mice are treated with ^{14}C-chloroform, the extent of hepatic necrosis parallels the amount of ^{14}C-label bound irreversibly to liver protein (Docks and Krishna, 1976; Brown et al., 1974; Ilett et al., 1973). Both necrosis and binding are potentiated by pretreatment of animals with phenobarbital, a known inducer of liver microsomal metabolism, and inhibited by pretreatment with the inhibitor piperonyl butoxide. Chloroform administration also decreases the level of liver glutathione in rats Pretreated with phenobarbital, further suggesting that a reactive metabolism is produced (Docks and Krishna, 1976; Brown et al., 1974). The results of in vitro studies with rat and mouse liver microsomes support the in vivo observations by establishing that ^{14}C-chloroform is metabolized to a reactive metabolite which binds covalently to microsomal protein (Bhooshan et al., 1977; Sipes et al., 1977; Uehleke and Werner, 1975; Ilett et al., 1973). This metabolic process is oxygen dependent and appears to be mediated by a cytochrome P_{450} which is inducible by phenobarbitol (Sipes et al., 1977; Uehleke and Werner, 1975; Ilett et al., 1973).

The demonstrations by Pohl et al. (1977) and Mansuy et al. (1977) of carbonyl chloride (phosgene) formation from chloroform by rat microsomal preparations suggest that phosgene may be the key causal agent for these toxic effects. The finding of Weinhouse and collaborators (Shah et al., 1979) that phosgene is also a reactive metabolic intermediate in the metabolism of carbon tetrachloride emphasizes basic similarities in the metabolism and toxicities of these two chloroalkanes. Figures 4-6 and 4-7 show for comparison the currently proposed pathways of metabolism of chloroform and carbon tetrachloride.

Figure 4-6 indicates that the initial step in the metabolism of chloroform involves the oxidation of the aliphatic carbon (H-C) to trichloromethanol by a phenobarbital inducible cytochrome P_{450} (Sipes et al., 1977; Uehleke and Werner, 1975; Ilett et al., 1973). This metabolic step has been suggested by Mansuy et al. (1977) and Pohl et al. (1977) as the precursor of phosgene formed by rat microsomes in vitro from chloroform. Phosgene was confirmed as a metabolite by reaction with cysteine to give 2-oxothiazolidine-4-carboxylic acid which was

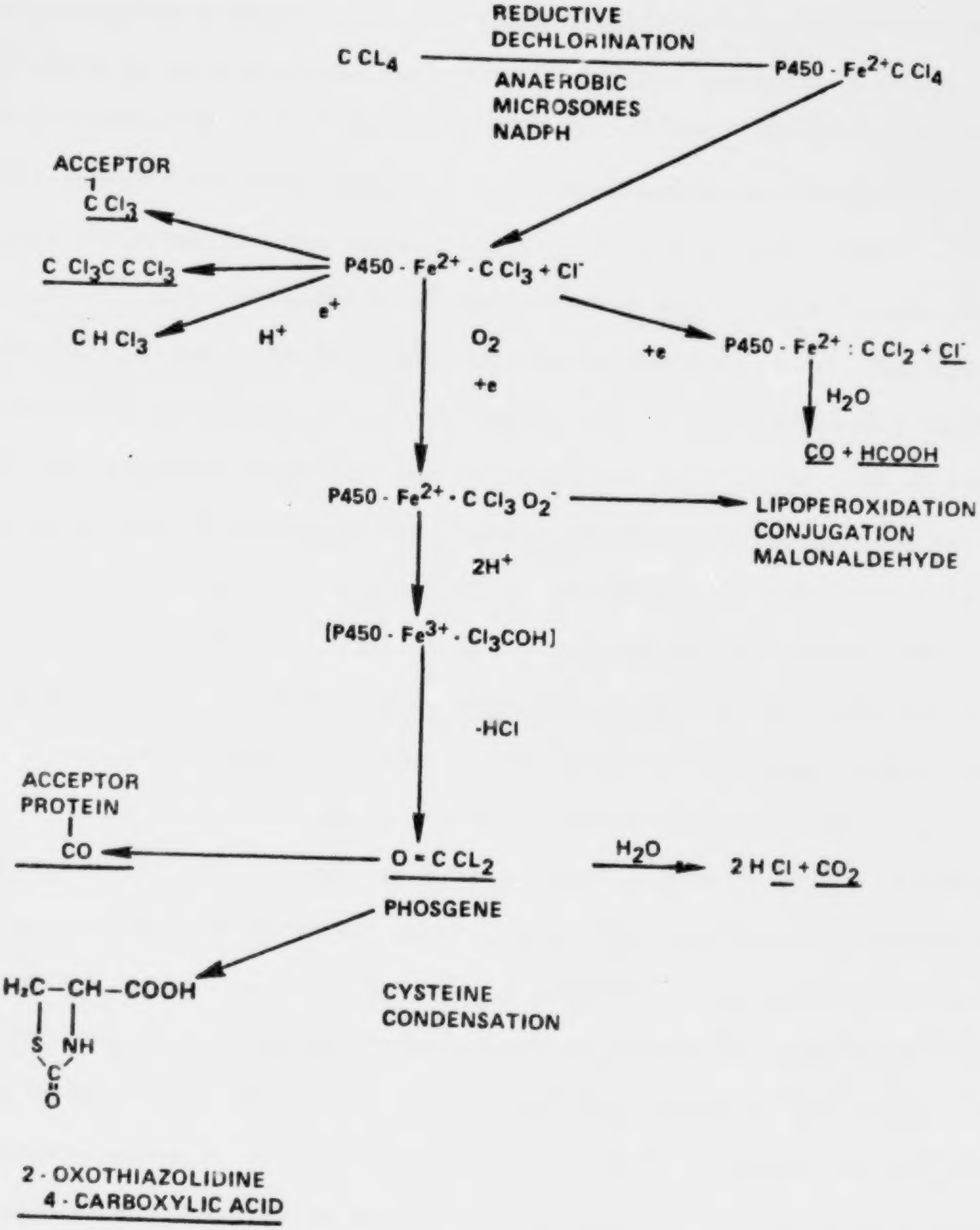

Figure 4-7. Metabolic pathways of carbon tetrachloride biotransformation. ($C\,Cl_4$ metabolites identified are underlined).

Source: Shah et al. (1979).

identified by GC-CIMS. Trichloromethanol is highly unstable and spontaneously dehydrochlorinates to produce phosgene (Seppelt, 1977). The electrophilic phosgene reacts with water to yield CO_2, a known metabolite of $CHCl_3$ in vitro (Rubinstein and Kanics, 1964; Paul and Rubinstein, 1963) and in vivo (Brown et al., 1974; Fry et al., 1972), with protein to form a covalently bound product (Pohl et al., 1977; Sipes et al., 1977; Uehleke and Werner, 1975; Brown et al., 1974; Ilett et al., 1973), or with cysteine (Pohl et al., 1977), and possibly with glutathione (Docks and Krishna, 1976; Brown et al., 1974). The finding that deuterated chloroform ($CDCl_3$) depletes glutathione in the livers of rats less than $CHCl_3$ supports this notion (Docks and Krishna, 1976).

The postulated oxidation of the C-H bond of chloroform by P_{450} to produce trichloromethanol which spontaneously yields phosgene is further supported by the observations of Pohl and Krishna (1978). These workers found that chloroform metabolism to phosgene by rat liver microsomes is oxygen and NADPH dependent, and inhibited by CO and SKF 525-A. Moreover, in the presence of cysteine and $^{18}O_2$ atmosphere, $^{18}O_2$ is incorporated into the 2-oxo position of 2-oxothiazolidine-4-carboxylic acid. Oxidative cleavage of the C-H bond appears to be the rate-determining step, since deuterium labeled chloroform ($CDCl_3$) is biotransformed into phosgene slower than $CHCl_3$; $CDCl_3$ appears also to be less hepatotoxic than $CHCl_3$. Pohl (1980) has further characterized the metabolism of chloroform in rat liver microsomes by measuring the covalent binding of $^{14}CHCl_3$ and C^3HCl_3 to microsomal protein. Chloroform does not appear to be activated by reductive dechlorination to the radical $\cdot CHCl_2$, because the ^3H-label does not bind to microsome protein as does the ^{14}C-label.

Figure 4-7 summarizes current knowledge of the biotransformation of carbon tetrachloride. The first step is a rapid reductive formation of the trichloromethyl ($\cdot CCl_3$) radical by complexing with one or more of the P_{450} cytochromes

(Shah et al., 1979; Poyer et al., 1978; Recknagel and Glende, 1973). This radical undergoes several reactions in addition to binding to lipids (Villarruel and Castro, 1975; Uehleke and Werner, 1975; Villarruel et al., 1975; Gordis, 1969; Reynolds, 1967) and protein (Uehleke et al., 1977; Uehleke and Werner, 1973), although not to nucleic acids (Uehleke et al., 1977; Uehleke and Werner, 1975; Reynolds, 1967). Anaerobically, the addition of a proton and electron yields chloroform (Glende et al., 1976; Uehleke et al., 1973; Fowler, 1969; Butler, 1961), dimerization to hexachloroethane (Uehleke et al., 1973; Fowler, 1969), or further reductive dechlorination to CO via the carbene, $:CCl_2$ (Wolf et al., 1977). Aerobically, the $\cdot CCl_3$ radical is oxidized by the P_{450} system to trichloromethanol (Cl_3COH), which is the precursor of phosgene (Cl_2CO) that Weinhouse and colleagues (Shah et al., 1979) have shown to be an intermediate in carbon tetrachloride metabolism by rat liver homogenates. Hydrolytic dechlorination of phosgene yields CO_2 (Shah et al., 1979).

Under normal physiological conditions (i.e., aerobic conditions), a minimal formation of chloroform might be expected to occur. Carbon tetrachloride yields a chloroform most readily _in vitro_ under anaerobic conditions and its formation is inhibited by oxygen (Uehleke et al., 1977; Glende et al., 1976). Shah et al. (1979) observed that chloroform does not compete successfully with carbon tetrachloride for initial binding to P_{450} cytochrome (Sipes et al., 1977; Recknagel and Glende, 1973). Wolf et al. (1977) also found that binding of chloroform to reduced cytochrome P_{450} was very slow compared to that of carbon tetrachloride.

Anders and coworkers (Anders et al., 1978; Ahmed et al., 1977) have shown, as they have for dihalomethanes, that trihalomethanes, including chloroform, also yield CO as a metabolite. Intraperitoneal administration of haloforms (1 to 4 mmoles/kg) to rats led to dose-dependent elevations in blood CO levels. Treatment of the rats with either phenobarbital (but not 3-methyl-cholanthrene) or SKF

525-A, respectively, increased or decreased metabolism to CO. The order of yield of CO from the iodoforms was greatest for iodoform > bromoform > chloroform for the same dose. Thus, chloroform was minimally metabolized to CO (i.e., to less than one-tenth of that for iodoform or bromoform). Similar findings were made by these workers with rat liver microsomes (Ahmed et al., 1977). Metabolism of the haloforms to CO by rat liver microsomes required NADPH, could proceed anaerobically but was increased 2-fold by O_2, was increased by pretreatment with phenobarbital and inhibited by SKF 525-A or $COCl_2$ pretreatment, and was stimulated by glutathione or cysteine addition in both anaerobic (3-fold) or aerobic (8-fold) conditions. These results suggested that haloforms were metabolized to CO via a cytochrome P_{450} dependent system. However, chloroform was a poor substrate compared to iodoform or bromoform, yielding <2% of its quantity of CO as formed from equimolar concentrations of these halomethanes. Wolf et al. (1977) also found that chloroform, to a very limited extent, was metabolized to CO by reduced rat P_{450} preparations. These workers investigated the spectral and biochemical interactions of a series of halogenated methanes with rat liver microsomes under anaerobic reducing conditions. Tetra- (e.g., CCl_4) and trihalogens (e.g., $CHCl_3$) all formed complexes with reduced cytochrome P_{450} with absorption peak at 460 to 465. A shift to 454 occurred with CO formation and subsequent complexing of CO to P_{450}. CO formation required NADPH, was higher in microsomes from phenobarbital and 3-methylcholanthrene-treated rats, and was not found at high oxygen concentrations (<8%). Figure 4-8 shows the relative rates of CO formation from carbon tetrachloride and other polyhalomethanes. Chloroform, in comparison to carbon tetrachloride, was a very poor reaction substrate, and binding of chloroform to reduced cytochrome P_{450} was extremely slow compared to that of carbon tetrachloride. Wolf et al. (1977) proposed the reduction sequence shown in Figures 4-6 and 4-8 for the reductive dechlorination of chloroform and of

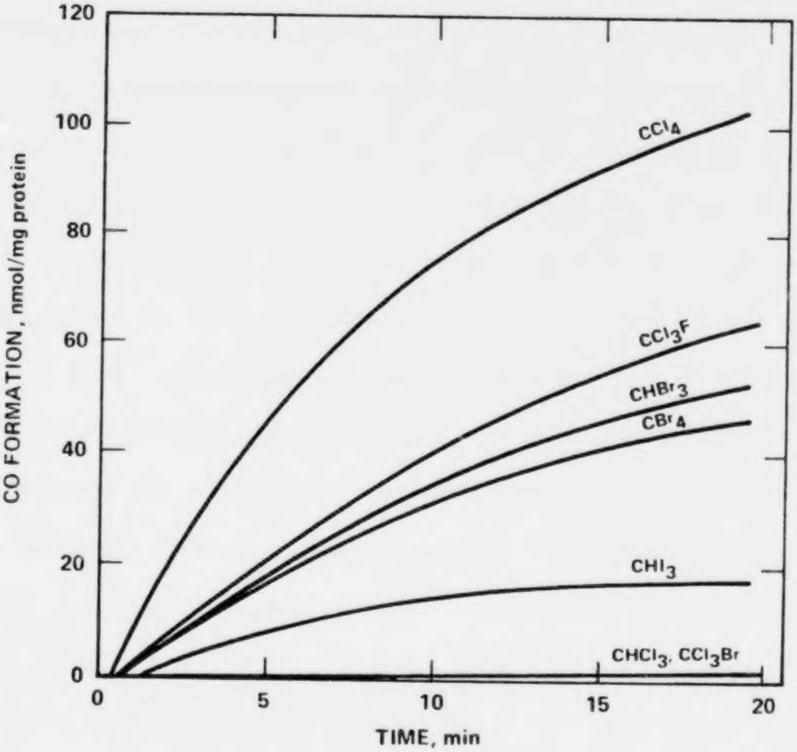

Figure 4-8. Rate of carbon monoxide formation after addition of various halomethanes to sodium dithionite-reduced liver microsomal preparations from phenobarbitol-treated rats. Note the low rate of metabolism of chloroform to CO compared to carbon tetrachloride.

Source: Wolf et al. (1977).

carbon tetrachloride to yield CO via a carbene (CCl_2) intermediate. The physiological importance of this pathway of metabolism appears to be more significant for carbon tetrachloride than for chloroform.

4.6. COVALENT BINDING TO CELLULAR MACROMOLECULES

4.6.1. Proteins and Lipids. Reactive intermediates of the metabolism of chloroform (phosgene, carbene, •Cl) and carbon tetrachloride (•CCl_3, phosgene, carbene, •Cl) that irreversibly bind to cellular macromolecules (covalent binding) are generally believed to result in an alteration of cellular integrity, which leads to centrolobular hepatic necrosis and renal proximal tubular epithelial damage. Chloroform, mole for mole, is generally accepted to be less hepatotoxic than carbon tetrachloride (Brown, 1972; Klaassen and Plaa, 1969; Plaa et al., 1958).

Chloroform in non-lethal doses produces renal damage in mice, dogs, and man; (Bhoosan et al., 1977; Pohl et al., 1977; Ilett et al., 1973; Klaassen and Plaa, 1966, 1967; Bennet and Whigham, 1964; von Oettingen, 1964; Conlon, 1963; Plaa et al., 1958; Culliford and Hewitt, 1957; Hewitt, 1956; Shubik and Ritchie, 1953) whereas, in experimental animals, carbon tetrachloride does not do so (Storms, 1973; Klaassen and Plaa, 1966; Plaa and Larson, 1965; Bennet and Whigham, 1964; Culliford and Hewitt, 1957; Hewitt, 1956; Shubik and Ritchie, 1953), although it does in man (New et al., 1962; Guild et al., 1958). To explain these species differences in toxicity as well as known intraspecies (Hill et al., 1975; Deringer et al., 1953; Shubik and Ritchie, 1953) and sex differences (Taylor et al., 1974; Ilett et al., 1973; Bennet and Whigham, 1964; Culliford and Hewitt, 1957; Hewitt, 1956; Deringer et al., 1953; Shubik and Ritchie, 1953; Eschenbrenner and Miller, 1945b), prevailing concepts implicate (1) differences in the rates of metabolism and organ system capacities for metabolism, which in turn determines the amount of irreversible macromolecular binding and (2) differences in the enzyme pathways for metabolism of the two haloalkanes (Figures 4-6 and 4-7).

Carbon tetrachloride, by a reductive dechlorination via complexing with reduced P_{450}, yields the trichloromethyl free radical ($\cdot CCl_3$) (Recknagel and Glende, 1973; Slater, 1972) (Figure 4-7), which can covalently bind to lipid and protein (Shah et al., 1979; Villarruel et al., 1975; Castro and Diaz Gomez, 1972; Reynolds, 1967), and can also initiate peroxidation of polyenoic fatty acids (Slater, 1972; Recknagel and Ghoshal, 1966). Chloroform does not appear to be activated to free radicals ($\cdot CCl_3$ or $\cdot CHCl_3$), but does bind covalently to liver lipid and protein (Sipes et al., 1977; Docks and Krishna, 1976; Uehleke and Werner, 1975; Brown et al., 1974; Ilett et al., 1973), and initiates lipid perioxidation in some circumstances (Koch et al., 1974; Ilett, 1973; Brown, 1972; Slater, 1972). Several investigators have shown that diene conjugates (products of lipoperoxidation) are not increased in vivo in normal rats when chloroform is inhaled or injected intraperitoneally (Brown et al., 1974; Brown, 1972; Klaassen and Plaa, 1969), but only when rats are pretreated with phenobarbital and the metabolism of chloroform is greatly enhanced (Brown et al., 1974; Brown, 1972). Studies in vitro show diene conjugation and malonaldehyde formation (an index of lipoperoxidation) by microsomes of phenobarbital pretreated rats were not increased but decreased by the addition of chloroform (Brown, 1972; Klaassen and Plaa, 1969), suggesting that with isolated microsomes, metabolism of chloroform is too small for sufficient quantities of reactive intermediates to accumulate and initiate lipoperoxidation. However, Rubinstein and Kanics (1964) found chloroform to be more rapidly metabolized by rat microsomal fractions than carbon tetrachloride (see above Table 4-11). These findings indicate that differences in

TABLE 4-12

Covalent Binding of Radioactivity From ^{14}C-Chloroform and ^{14}C-Carbon Tetrachloride in Microsomal Incubation *In Vitro*[a]

	Incubation Condition	Microsomal[b] Protein	Microsomal[b] Lipid	To Added serum albumin
		nmol/mg in 60 minutes		
$^{14}C-CCl_4$	N_2	20.0	76.0	1.4
$^{14}C-CHCl_3$	N_2	5.1	4.1	0.9
	O_2	8.5	7.0	1.7

[a] Source: Uehleke et al., 1977

[b] Microsomes from phenobarbital pretreated rabbits

metabolic activation [carbon tetrachloride to produce free radicals (Figure 4-7), but chloroform primarily to phosgene (Figure 4-6)] explain the greater potential of carbon tetrachloride for initiating lipoperoxidation. Table 4-11 shows the data of Uehleke et al. (Uehleke et al., 1977; Uehleke and Werner, 1975) for the covalent binding of rabbit microsomes following incubation with ^{14}C-labeled chloroform and carbon tetrachloride. Both protein and lipid binding of ^{14}C-radioactivity are 4-fold and 20-fold, respectively, more extensive for carbon tetrachloride than chloroform; lipids are labeled preferentially by carbon tetrachloride but are not by chloroform. Furthermore, covalent binding from chloroform metabolism occurs mainly with anaerobic conditions (a minor metabolic pathway) and is not greatly increased with aerobic metabolism, the major pathway for metabolism of chloroform, which is O_2 dependent (Figure 4-6).

Covalent binding occurs preferentially to lipids and proteins of the endoplasmic reticulum proximate to P_{450} system for metabolism. However, considerable covalent binding from chloroform metabolites occurs in other cell fractions of liver and kidney, particularly to mitochondria (Uehleke and Werner, 1975; Hill et al., 1975). Hill et al. (1975) found when C57BL male mice were injected interperitoneally with 0.07 mℓ/kg ^{14}C-chloroform in oil and sacrificed 12 hours later, that in the liver, 50% of the radioactivity was irreversibly bound to microsome, 23% to mitochondria, 25% to cytosol, and <2% to nuclei; for kidney, 38% of radioactivity was bound to microsome, 39% to mitochondria, 22% to cytosol, and <2% to nuclei. A similar distribution was found in male NMRI mice by Uehleke and Werner (1975), who observed minimal binding to microsomal RNA but significant binding to nicotine-adenine nucleotides. The data of Ilett et al. (1973), shown in Figure 4-9, demonstrates that in C57 BL/6 mice, the amount of covalent binding in liver and kidney microsomal fractions increases proportionally with the chloroform dose.

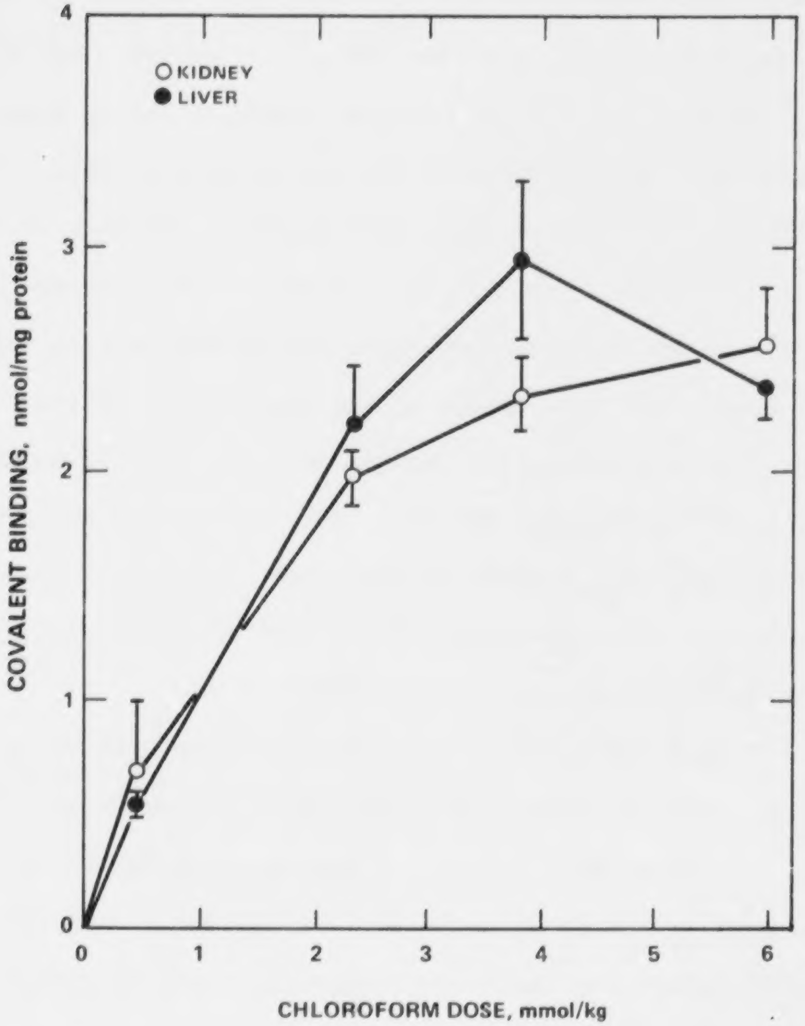

Figure 4-9. Effect of increasing dosage of i.p.-injected ^{14}C-chloroform on extent of covalent binding of radioactivity *in vivo* to liver and kidney proteins of male mice 6 hours after administration.

Source: Ilett et al. (1973).

4.6.1.1. GENETIC STRAIN DIFFERENCE -- Hill et al. (1975) described in mice two genetic variations in chloroform toxicity paralleling genetic differences in covalent binding in liver and kidney. In one inbred strain (DBA/2), the male animals were 4 times more sensitive to the lethal effects of oral doses of chloroform (LD_{50} of 0.08 mℓ/kg) than the second strain (C57 BL/6, LD_{50} of 0.33 mℓ/kg). Males of the F_1 hybrid strain (B6D2F_1/J) had an intermediate LD_{50} of 0.2 mℓ/kg, midway between those of the two parental strains. The susceptibility of DBA mice was related to a dose-dependent necrosis of the proximal convoluted renal tubules. However, mice of all three genotypes that received >0.17 mℓ/kg chloroform exhibited both renal tubular necrosis and hepatic centrolobular necrosis. Males and females of the same strain exhibited similar dose thresholds to hepatic damage, but females died of chloroform-induced hepatic damage without developing renal lesions. This sex-related absolute difference is dependent on androgen profile of the mice; testosterone-treated females become sensitive to renal toxicity (Bennet and Whigham, 1964; Culliford and Hewitt, 1957; Eschenbrenner and Miller, 1945b).

Table 4-12 shows the extent of covalent binding in liver and kidney of these three strains after a single intraperitoneal injection of ^{14}C-chloroform (0.07 mℓ/kg) to the males. Kidney homogenates from DBA/2J male mice, more sensitive to renal necrosis, contained more than 2-fold as much radioactivity as those from resistant C57BL/6J; covalent binding in the F_1 hybrid was intermediate, as expected. A significant difference was also noted in labeling of kidney subcellular fractions. While all subcellular fractions of susceptible male DBA mice were labeled to a greater extent than F_1 or C57BL strains, the greatest increase was in labeling of the mitochondrial fraction.

TABLE 4-13

Mouse Strain Difference in Covalent Binding of Radioactivity From ^{14}C-Chloroform[a,b]

Tissue	Specific Activity Relative to C57 BL		
	DBA	F_1	C57BL
		Tissue homgenates	
Liver	0.82	0.96	1.00
Kidney	2.41	1.64	1.00
		Subcellular fractions	
Liver			
Nuclei	0.67	0.76	1.00
Mitochondria	1.14	1.14	1.00
Microsomes	0.64	0.73	1.00
Cell sap	0.98	1.09	1.00
Kidney			
Nuclei	2.20	1.67	1.00
Mitochondria	3.67	1.97	1.00
Microsomes	1.74	1.44	1.00
Cell sap	1.65	1.23	1.00

[a] Source: Hill et al., 1975

[b] Adult male mice of each genotype given ^{14}C-chloroform (0.07 mℓ/kg) intraperitoneally and sacrified 12 hours later. Genotype comparisons are given as ratio of radioactivity to C57BL = 1.

In the liver, the distribution of covalent binding was generally opposite to that observed in the kidneys (Table 4-12), but neither liver homogenates nor subcellular fractions showed significant strain differences.

4.6.1.2. SEX DIFFERENCE -- Kidneys of male mice are known to covalently bind more ^{14}C-chloroform radioactivity than do those of females, but females bind more in the liver than males (Taylor et al., 1974; Ilett et al., 1973) (see Tables 4-7 and 4-13). Table 4-14 shows that pretreatment of male mice with phenobarbital increases covalent binding in the liver but not in the kidney (Ilett et al., 1973). A similar observation has been made by Kluwe et al. (1978) in male mice. They found that phenobarbital increased liver but not kidney microsomal activity; 3-methylcholanthrene, dioxin, and PCGs increased both liver and kidney microsomal enzyme activities. From the renal and hepatic toxicity profile to chloroform displayed by mice treated with these various inducers, these investigators concluded that the chloroform metabolite(s) responsible for hepatic damage is probably generated in the liver, and the metabolite(s) responsible for renal damage is generated in the kidney.

4.6.1.3. INTER-SPECIES DIFFERENCE -- In addition to intra-species strain (mice) differences in covalent binding noted above, Uehleke and Werner (1975) have also observed an apparent inter-species difference. Figure 4-10 shows the *in vitro* binding of radioactivity from ^{14}C-chloroform by microsomal preparations from rat, mouse, rabbit, and man. Human and rabbit microsomes have the highest rate of covalent binding from chloroform, with the mouse followed by the rat considerably lower. Inter-species differences in the covalent binding rates for carbon tetrachloride were small. These species differences in binding of chloroform metabolites to protein and lipid *in vitro* do not, however, parallel the

TABLE 4-14

In Vivo Covalent Binding of Radioactivity From $^{14}CHCl_3$ in Liver and Kidney of Male and Female Mice (C57BL/6)[a,b]

	Covalent Binding of ^{14}C-Chloroform nmoles/mg protein ± S.E.	
	Male	Female
Liver	2.92 ± 0.35	3.66 ± 0.39
Kidney	2.34 ± 0.16	0.39 ± 0.02

[a] Source: Ilett et al., 1973

[b] Mice were sacrificed 6 hours after intraperitoneal administration of 3.72 nmoles/kg of $^{14}CHCl_3$.

TABLE 4-15

In Vitro Covalent Binding of Radioactivity from $^{14}CHCl_3$ to Microsomal Protein from Liver and Kidney of Male and Female Mice (C57BL/6)*

	Pretreatment	Covalent Binding of ^{14}C-Chloroform p moles/mg protein/5 minutes ± SEM	
		Liver	Kidney
Male	NA	572 ± 54	44.6 ± 4.1
Male	Phenobarbital	1454 ± 143	41.0 ± 3.2
Female	NA	419 ± 20	14.6 ± 2.5

*Source: Ilett et al., 1973.

NA = Not applicable

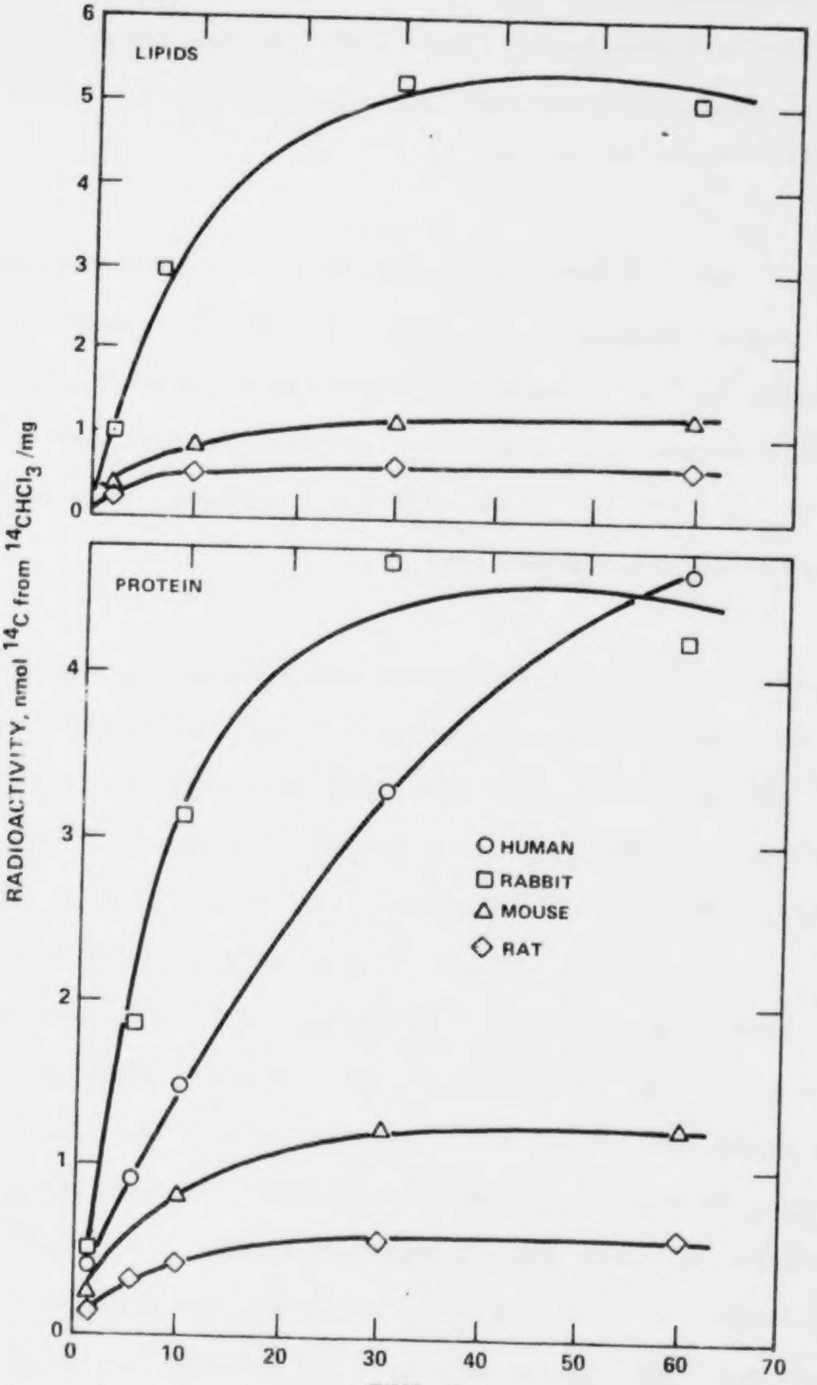

Figure 4-10. Comparison of irreversible binding of radioactivity from ^{14}C-CHCl$_3$ to protein and lipid of microsomes from normal rabbit, rat, mouse, and human liver incubated *in vitro* at 37°C in O_2.

Source: Uehleke and Werner (1975).

species differences in metabolism of chloroform in vivo, as measured by the conversion of chloroform to CO_2 (Table 4-9); in vivo, the mouse has the greatest capacity to metabolize chloroform (80%), followed by rats (65%), nonhuman primates (20%), and man (30 to 50%).

4.6.1.4. AGE DIFFERENCE -- Uehleke and Werner (1975) have shown that irreversible protein binding of radioactivity from ^{14}C-chloroform and ^{14}C-carbon tetrachloride to liver microsomes of newborn rats (18 hours old) is low compared to that of microsomes from adult rats (32 days); however, the binding was shown to be proportional to P_{450} content of the microsomes, which was proportionally low in microsomes from newborn rats.

4.6.2. Nucleic Acids. P_{450} systems activate chloroform and carbon tetrachloride in vivo and in vitro to reactive metabolites that extensively covalently bind to proteins and lipids, but do so only minimally to nucleic acid (Uehleke et al., 1977; Wolf et al., 1977; Uehleke and Werner, 1975; Fowler, 1969; Reynolds, 1967), unlike many other carcinogens that bind DNA. Reitz et al. (1980) measured DNA alkylation in liver and kidneys of mice after an oral dose of 240 mg/kg ^{14}C-chloroform (specific activity not given) and found values of 3×10^{-4} and 1×10^{-4} mol % for liver and kidney DNA respectively. These workers judged that chloroform has very little direct interaction with DNA when compared to known carcinogens, as reported in the literature for dimethylnitrosamine (3.5×10^{-1} mol % alkylation, liver DNA), dimethylhydralazine (2.6×10^{-2}, colon DNA) and N-methyl-N-nitrosourea (1.5×10^{-1}, brain DNA) but given by parenteral routes (Pegg and Hui, 1978; Cooper et al., 1978; Kleihues and Margison, 1974). The failure of chloroform or carbon tetrachloride reactive species to significantly bind DNA has been ascribed to their short half-life compared to epoxides, and to their lack of nuclear penetration. Recently, however, Diaz Gomez and Castro (1980) have shown that highly purified rat liver nuclear preparations are able to anaerobically activate carbon tetrachloride, and to aerobically activate chloroform to reactive metabolites that bind to nuclear lipids and proteins. Their data, given in Table 4-15, show that activity in nuclear preparations is smaller than in microsomes, but within the same order of magnitude. These results might be relevant to the hepatocarcinogenic effects of chloroform and carbon tetrachloride in mice and rats, since the nuclear targets (DNA, RNA, nuclear proteins) are in the immediate neighborhood sites of activation, thus, making unnecessary the present assumption that the highly reactive intermediates ($\cdot CCl_3$, phosgene, malonaldehyde, or carbene), produced at the endoplasmic reticulum, must travel to the nucleus.

4.6.3. Role of Phosgene. Phosgene is a prominent intermediate of both chloroform and carbon tetrachloride metabolisms (Figures 4-6, 4-7). It is known to be highly reactive and toxic to cells and tissues (Pawlowsky and Frosolono, 1977), and its two highly reactive chlorines suggest that it could act on cellular macromolecules similar to bifunctional alkylating agents. Reynolds (1967) showed that ^{14}C-phosgene, given to intact rats, labeled liver protein (and lipids to a smaller extent). The pattern of labeling was quite different from that of ^{14}C-carbon tetrachloride and more similar to ^{14}C-chloroform. Moreover, ^{36}Cl-carbon tetrachloride radioactivity was also stably incorporated into liver lipid and protein, pointing to the $\cdot CCl_3$ radical rather than phosgene as the reactive form for carbon tetrachloride that labels lipid. Cessi et al. (1966) also reported that ^{14}C-phosgene labeled terminal amino group of polypetides in a manner similar to *in vivo* protein labeling produced by carbon tetrachloride.

TABLE 4-16

Covalent Binding of Radioactivity from ^{14}C-Chloroform and ^{14}C-Carbon Tetrachloride in Rat Liver Nuclear and Microsomal Incubation *In Vitro**

	Incubation Condition	Protein	Lipid
		p mol/mg ± S.D.	
$^{14}C-CCl_4$	N_2		
Nuclear		21.9 ± 2.5	147 ± 12
Microsomal		50.3 ± 4	190 ± 11
$^{14}C-CHCl_3$	O_2		
Nuclear		27.0 ± 3	20 ± 3
Microsomal		68.0 ± 9	57 ± 8

*Source: Diaz Gomez and Castro, 1980

4.6.4. Role of Glutathione. Ekstrom and Hogberg (1980) found that chloroform, in freshly isolated rat liver cells, induced depletion of cellular glutathione. Brown et al. (1974) demonstrated that exposure of rats to an atmosphere of 0.5% chloroform for 2 hours markedly decreased glutathione (GSH) in the liver when the animals were pretreated with phenobarbital to stimulate metabolism. GSH liver content of untreated rats was not decreased. Phenobarbital pretreatment has been shown to markedly potentiate toxicity of both chloroform and carbon tetrachloride in rats (Docks and Krishna, 1976; Cornish et al., 1973; McLean, 1970; Scholler, 1970). However, it has not been possible to detect a decrease in the liver glutathione levels following administration of carbon tetrachloride or trichlorobromomethane (Docks and Krishna, 1976; Boyland and Chasseaud, 1970). Sipes et al. (1977) have shown that the addition of GSH liver microsomes from phenobarbital pretreated rats incubated in vitro with ^{14}C-labeled halocarbons, chloroform, carbon tetrachloride, and trichloromomethane, inhibited covalent binding ≈80% for all three compounds. Their results, given in Table 4-16, also show the effects of anaerobic and aerobic conditions on covalent binding. The reduction in binding of chloroform by an atmosphere of N_2 suggests that its bioactivation is mediated by a cytochrome P_{450} oxidative pathway to phosgene (Figure 4-6), while the enhanced binding of carbon tetrachloride in N_2 reflects P_{450} mediated reductive pathways (Figure 4-7) and formation of free radical. These investigators suggest that in phenobarbital-treated animals, chloroform depletes liver GSH by the formation of conjugate between the reactive intermediate phosgene and GSH (Docks and Krishna, 1976; Brown et al., 1974). In the case of carbon tetrachloride, they suggest that GSH addition in vitro (Table 4-16) also conjugates with the phosgene metabolite of carbon tetrachloride produced when incubated in air, but, in addition, GSH decreases the levels of $\cdot CCl_3$ by reducing the free radical to chloroform. In vivo, it is postulated that

TABLE 4-17

Effect of Glutathione, Air, N_2 or CO: O_2 Atmospere on the *In Vitro* Covalent Binding of C Cl_4, $CHCl_3$ and C Br Cl_3 to Rat Liver Microsomal Protein[a]

Incubation Conditions	Substrate		
	CCl_4[b]	$CHCl_3$	$CBrCl_3$
	p moles ^{14}C-bound/mg microsomal protein/minute		
Air	97 ± 10	59 ± 5	1456 ± 66
N_2	310 ± 51	21 ± 1	1370 ± 143
$CO:O_2$(8:2)	18 ± 1	20 ± 1	853 ± 62
SKF 525 A (0.5 mM), Air	109 ± 5	7 ± 7	2105 ± 159
Glutathione, Air	17 ± 2	15 ± 2	218 ± 25
NADPH omitted, Air	6 ± 1	3 ± 0	65 ± 13

[a] Source: Sipes et al., 1977

[b] ^{14}C-labeled substrate is a final concentration of 1×10^{-3}M incubated at 37°C. Microsomes were from phenobarbital pretreated rats.

the oxidized glutathione may be reduced back to reduced GSH by glutatione reductase; this would explain the lack of fall of liver GSH content with carbon tetrachloride in vivo (Gillette, 1972). Thus, the toxic effects of chloroform and carbon tetrachloride may be mediated through different mechanisms of covalent binding, and GSH may play different roles for these chlorocarbons in preventing covalent binding of reactive intermediates of metabolism.

Chloroform and carbon tetrachloride are known to cause greater liver damage in fasted animals than in fed animals (Diaz Gomez et al., 1975; Jaeger et al., 1975; Krishnan and Stenger, 1966; Goldschmidt et al., 1939; Davis and Whipple, 1919). For chloroform, a decreased content of hepatic GSH from fasting has been postulated to be responsible for the increased susceptibility of fasted mice (Docks and Krishna, 1976; Brown et al., 1974). Nakajima and Sato (1979) have recently offered an additional explanation. These investigators studied the metabolism of the chlorocarbons in vitro with microsomes from livers of fasted rats, and found that the disappearance of chloroform from incubation increased 3-fold for a 24 hour fast, although fasting produced no significant increase in the microsomal protein and cytochrome P_{450} liver contents (Table 4-17). Similar results were obtained for carbon tetrachloride. These observations suggest that the increased toxicity of chloroform and carbon tetrachloride from food deprivation may be due not only to decreased GSH, but also to a greater production of reactive intermediates and covalent binding to cellular macromolecules.

SUMMARY

At ambient temperatures, chloroform is a volatile liquid with high lipid solubility and appreciable solubility in water. Hence, chloroform is readily absorbed into the body through the lungs and intestinal mucosa; the portals of

TABLE 4-18

Effects of 24-Hour Food Deprivation on Chloroform and Carbon Tetrachloride In Vitro Microsomal Metabolism, Protein, and P-450 Liver Contents of Rats*

	Male			Female		
	Fed	Fasted	Ratio	Fed	Fasted	Ratio
			Metabolism, nmole/g/min			
Chloroform	19.7 ± 2.6	55.1 ± 7.5	2.8	15.3 ± 6.8	39.3 ± 2.5	2.6
Carbon tetrachloride	1.9 ± 0.2	5.9 ± 0.8	3.1	1.1 ± 0.5	4.5 ± 0.3	4.1
			Protein content, mg/kg liver			
	27.7 ± 3.7	23.0 ± 2.7	NR	22.5 ± 1.5	23.7 ± 1.7	NR
			P-450, nmol/mg protein			
	0.842 ± 0.123	0.823 ± 0.03	NR	0.638 ± 0.051	0.673 ± 0.044	NR

*Source: Nakajima and Sato, 1979

NR = Not reported

entry with exposure from air, water and food. Few data are available on the pharmacokinetics of absorption and excretion of chloroform in man, particularly at the low exposure concentrations expected in ambient air and drinking water. However, studies show absorption from the gastrointestinal tract in man, monkeys, rats and mice is rapid and complete, occurring by first-order passive absorptive processes. A dose-dependent first-pass effect with pulmonary elimination of unchanged chloroform occurs with oral ingestion in man, thus decreasing the amount of chloroform reaching the systemic circulation. In rats, the kinetics of peroral absorption are also influenced by the dosing vehicle; the absorption rate is decreased for chloroform given in corn oil vehicle as compared to an aqueous solution. Pulmonary uptake and elimination occur also by first-order diffusion processes with three distinct components with rate constants corresponding to tissue loading or desaturation of at least three major body compartments. Half-times in man have been found to be approximately 14-30 minutes, 90 minutes and 24-36 hours, respectively. The longest half-time is associated with the lipids and the adipose tissue compartment. During inhalation exposure, at equilibrium with inspired air concentration, the blood/air partition coefficient is about 8 at 37°C and the adipose tissue/blood partition coefficient is 280 at 37°C. The quantity of chloroform absorbed is dependent also on body weight and fat content of the body.

Tissue distribution of chloroform is consistent with its lipophilic nature and modest water solubility. This chloroalkane readily crosses the blood brain and placental barriers and distributes into breast milk. Concentrations occurring in all major tissue organs are dose related to inspired air concentrations or to oral dosage. Relative tissue concentrations occur in the order of adipose tissue > brain > liver > kidney > blood.

Elimination of chloroform from the body occurs by two major and parallel occurring processes: 1) pulmonary elimination of unchanged chloroform by first order kinetics, and 2) metabolism of chloroform. Chloroform is metabolized in the liver, and to a lesser extent in the kidneys and other tissues. Metabolism is dose-dependent and saturable, with a greater proportion of small doses being metabolized. There are striking differences in the pharmacokinetics and quantitative metabolism of chloroform in man as compared to other animals. For large steady-state body burdens, 30-40% is metabolized by man, 20% by the nonhuman primate, > 65% by the rat, and > 85% by the mouse. Metabolism produces phosgene and other putative reactive metabolites that covalently bind extensively to cellular lipids and proteins, although not significantly to DNA or other nucleic acids. The intensity of metabolite binding and organ localization parallel the acute cellular toxicity of chloroform in liver and kidney observed in experimental animals. Both binding and toxicity are highly dependent on animal species and genetic strain, as well as on sex and age. An additional variable is the tissue level of reduced glutathione which plays an important role in protecting against both binding and toxicity. Conversely, inducers of hepatic and renal P450 metabolizing systems increase binding and toxicity.

4.8 REFERENCES

Ahmed, A.E., V.L. Kubic and M.W. Anders. 1977. Metabolism of haloforms to carbon monoxide. I. In vitro studies. Drug Metab. Dispos. 5: 198-204.

Anders, M.W., J.L. Stevens, R.W. Sprague, Z. Shaath and A.E. Ahmed. 1978. Metabolism of haloforms to carbon monoxide. II. In vivo studies. Drug Metab. Dispos. 6: 556-560.

Bellar, T.A., J.J. Lichtenburg and R.C. Kroner. 1974. Occurrence of organohalides in chlorinated drinking waters. Amer. Water Works Assoc. Jour. 66: 703-706.

Bennet, R.A. and A. Whigham. 1964. Chloroform sensitivity of mice. Nature. 204: 1328.

Bhooshan, B., L.R. Pohl and G. Krishna. 1977. A biochemical mechanism for chloroform activation. Fed. Proc. (Abstr). 36: 396.

Boyland, E. and L.F. Chasseaud. 1970. The effect of some carbonyl compounds on rat liver glutathione levels. Biochem. Pharmacol. 19: 1526-1528.

Brown, D.M., P.F. Langley, D. Smith and D.C. Taylor. 1974a. Metabolism of chloroform. I. The metabolism of ^{14}C-chloroform by different species. Xenobiotica. 4: 151-163.

Brown, B.R., K.G. Sipes and A.M. Sagalyn. 1974b. Mechanisms of acute hepatic toxicity: Chloroform, halothane and glutathione. Anesthesiol. 41: 554-561.

Brown, S.S. 1972. In Foreign compound metabolism in mammals. vol. 2. Mathway, D.C., ed. London, The Chemical Society. p. 461.

Butler, T.C. 1961. The reduction of carbon tetrachloride in vivo and reduction of carbon tetrachloride to chloroform in vitro by tissues and tissue constituents. Jour. Pharmacol. Exp. Ther. 134: 311-319.

Castro, J.A. and M.I. Diaz Gomez. 1972. Studies on irreversible binding of $^{14}C\text{-}CHCl_3$ to microsomal lipids in rats under varying experimental conditions. Toxicol. Appl. Pharmacol. 23: 541-552.

Cessi, C., C. Columbini and L. Mameli. 1966. The reaction of liver proteins with a metabolite of carbon tetrachloride. Biochem. Jour. 101: 46c.

Chenoweth, M.B., D.N. Robertson, D.S. Erly and K. Goekhe. 1962. Blood and tissue levels of ether, chloroform, halothane and methoxyflurane in dogs. Anesthesiol. 23: 101-106.

Chiou, W.L. 1975. Quantitation of hepatic and pulmonary first-pass effects and its implications in pharmacokinetic study. I. Pharmacokinetics of chloroform in man. J. Pharmac. Biopharmaceut. 3: 193-201.

Cohen, E.N. and W. Hood. 1969. Application of low-temperature autoradiography studies of the uptake and metabolism of volatile anesthetics in the mouse. Anesthesiol. 30: 306-314.

Conkle, J.P., B.J. Camp and B.E. Welch. 1975. Trace composition of human respiratory gas. Arch. Environm. Health. 30: 290-295.

Conlon M.F. 1963. Addiction to chlorodyne. Brit. Med. Jour. 2: 1177-1178.

Cooper, H.K., J. Buecheler and P. Kleihues. 1978. DNA alkylation in mice with genetically different susceptibility to 1,2-dimethylhydrazine-induced colon carcinogenesis. Cancer Res. 38: 3063-3065.

Cornish, H.H., B.P. Ling and M.L. Barth. 1973. Phenobarbital and organic solvent toxicity. Amer. Ind. Hyg. 34: 487-492.

Culliford, D. and H.B. Hewitt. 1957. The influence of sex hormone status on the susceptibility of mice to chloroform-induced necrosis of renal tubules. Jour. Endocr. 14: 381-393.

Davis, N. and C. Whipple. 1919. The influence of fasting and various diets on the liver injury effected by chloroform anesthesia. Arch. Int. Med. 23: 612-633.

Deringer, M.K., T.B. Dunn and W.E. Heston. 1953. Results of strain C3H mice to chloroform. Proc. Soc. Exp. Biol. Med. 83: 474-479.

Diaz Gomez, M.I. and J.A. Castro. 1980. Nuclear activation of carbon tetrachloride on chloroform. Res. Comm. Chem. Path. Pharmacol. 27: 191-194.

Diaz Gomez, M.I., C.R. DeCastro, E.C. DeFerreyra, N. D'Acosta, O.M. DeFerros and J.A. Castro. 1975. Mechanistic studies on carbon tetrachloride hepatotoxicity in fasted and fed rats. Toxicol. Appl. Pharmacol. 32: 101-108.

Dilley, J.V., N. Chernoff, D. Kay, N. Winslow and G.W. Newell. 1977. Inhalation teratology studies of five chemicals in rats. Toxicol. Appl. Pharmacol. 41: 196.

Docks, E.L. and G. Krishna. 1976. The role of glutathione on chloroform-induced hepatoxicity. Exp. Mol. Pathol. 24: 13-22.

Dowty, B.J., D.R. Carlisle and L.L. Laseter. 1975. New Orleans drinking water sources tested by gas chromatography-mass spectrometry. Occurrence and origin of aromatics and halogenated aliphatic hydrocarbons. Environm. Sci. Technol. 9: 762-765.

Droz, P.O. and J.G. Fernandez. 1977. Effect of physical workload on retention and metabolism of inhaled organic solvents. A comparative theoretical approach and its application with regards to exposure monitoring. Int. Arch. Occup. Environ. Hlth. 38: 231-246.

Eger, E.I. 1963. Mathematical model of uptake and distribution. In: Papper, E.M. and Kitz, R.J. eds. Uptake and Distribution of Anesthetic Agents. McGraw-Hill, New York. pp. 72-103.

Ekstrom, T. and J. Hogberg. 1980. Chloroform induced glutathione depletion and toxicity in freshly isolated hepatocytes. Biochem. Pharmacol. 29: 3059-3064.

Eschenbrenner, A.B. and E. Miller. 1945a. Induction of hepatomas in mice by repeated oral administration of chloroform with observations on sex differences. Jour. Natl. Cancer Inst. 5: 251-255.

Eschenbrenner, A.B. and E. Miller. 1945b. Sex differences in kidney morphology and chloroform necrosis. Science. 102: 302-303.

Feingold, A. and D.A. Holaday. 1977. The pharmacokinetics of metabolism of inhalation anaesthetics. Brit. Jour. Anaesth. 49: 155-162.

Fiserova-Bergerova, V., J. Vlach and J.C. Cassady. 1980. Predictable "individual differences" in uptake and excretion of gases and lipid soluble vapours simulations study. Br. J. Ind. Med. 37: 42-49.

Fiserova-Bergerova, V. and D.A. Holaday. 1979. Uptake and clearance of inhalation anesthetics in man. Drug. Metab. Rev. 9: 43-60.

Fiserova-Bergerova, V., J. Vlach and K. Singhal. 1974. Simulation and prediction of uptake, distribution, and exhalation of organic solvents. Br. J. Ind. Med. 31: 45-52.

Fishbein, L. 1979. Potential halogenated industrial carcinogenic and mutagenic chemicals. II. Halogenated saturated hydrocarbons. Sci. Total Environm. 11: 163.

Fowler, S.J.L. 1969. Carbon tetrachloride metabolism in the rabbit. Brit. Jour. Pharmacol. 37: 733-737.

Fry, J., T. Taylor and D.F. Hathaway. 1972. Pulmonary elimination of chloroform and its metabolite in man. Arch. Int. Pharmacodyn. 196: 98-111.

Geigy Scientific Tables. 1973. Diem, K. and C. Lentner, eds. 7th ed. Ciba-Ceigy, Basle.

Gettler, A.O. 1934. Medicolegal aspects of deaths associated with chloroform or ether. Amer. Jour. Surg. 26: 168-174.

Gettler, A.O. and H. Blume. 1931. Chloroform in the brain, lungs and liver. Quantitative recovery and determination. Arch. Pathol. 11: 554-560.

Gillette, J.R. 1972. Pharmacology and the future of man. In: Proceeding of the 5th Intern. Cong. Pharmacol. San Francisco, Karger, Basel.

Glende, E.A. Jr., A. M. Hruszkewycz and H.O. Recknagel. 1976. Critical role of lipid peroxidation in carbon tetrachloride-induced loss of aminopyridine demethylase, cytochrome P450 and glucose 6-phosphatase. Biochem. Pharmacol. 25: 2163-2170.

Goldschmidt, S., N.M. Vars and I. Ravdin. 1939. The influence of the foodstuffs upon the susceptibility of the liver to injury by chloroform and the probable mechanism of their action. Jour. Clin. Invest. 18: 277-289.

Gordis, E. 1969. Lipid metabolites of carbon tetrachloride. Jour. Clin. Invest. 48: 203-209.

Guild, W.R., J.V. Young and J.P. Merrill. 1958. Anuria due to carbon tetrachloride intoxication. Ann. Int. Med. 48: 1221-1227.

Hewitt, H.B. 1956. Renal necrosis in mice after accidental exposure to chloroform. Brit. Jour. Exp. Path. 37: 32-39.

Hill, R.N., T.L. Clemens, D.K. Liu, E.S. Vesell and W.D. Johnson. 1975. Genetic control of chloroform toxicity in mice. Science. 190: 159-160.

Ilett, K.F., W.D. Reid, I.G. Sipes and G. Krishna. 1973. Chloroform toxicity in mice: Correlation of renal and hepatic necrosis with covalent binding of metabolites to tissue macromolecules. Exp. Mol. Pathol. 19: 215-229.

Jaeger, R.J., R.B. Connolly and S.D. Murphy. 1975. Short-term inhalation toxicity of halogenated hydrocarbons. Effects on fasting rats. Arch. Environm. Health. 30: 26-30.

Jakobson, I., J.E. Wahlberg, B. Holmberg and G. Johansson. 1982. Uptake via the blood and elimination of 10 organic solvents following epicutaneous exposure to anesthetized guinea pigs. Toxicol. Appl. Pharmacol. 63: 181-187.

Kimura, E.T., D.M. Ebert and B.W. Dodge. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol. Appl. Pharmacol. 19: 699-704.

Klaassen, C.D. and G.L. Plaa. 1969. Comparison of the biochemical alterations elicited in livers from rats treated with carbon tetrachloride, chloroform, and metahylchloroforms. Biochem. Pharmacol. 18: 2019-2027.

Klaassen, C.D. and G.L. Plaa. 1967. Relative effects of various chlorinated hydrocarbons on liver and kidney function in mice. Toxicol. Appl. Pharmacol. 10: 119-131.

Klaassen, C.D. and C.L. Plaa. 1966. Relative effects of various chlorinated hydrocarbons on liver and kidney function in mice. Toxicol. Appl. Pharmacol. 9: 139-151.

Kleihues, P. and G.P. Margison. 1974. Carcinogenicity of N-methyl-N-nitrosurea. Possible role of excision repair of O^6-methylguanine from DNA. J. Nat. Cancer Inst. 53: 1839-1841.

Kluwe, W.M., K.M. McCormack and J.B. Hook. 1978. Selective modification of the renal and hepatic toxicities of chloroform by induction of drug metabolizing enzyme systems in kidney and liver. Jour. Pharmacol. Exp. Therap. 207: 566-573.

Koch, R.R., E.A. Glende and R.O. Recknagel. 1974. Hepatotoxicity of bromotrichloromethane-bond dissociation energy and lipoperoxidation. Biochem. Pharmacol. 23: 2907-2915.

Krishnan, N. and R. Stenger. 1966. Effects of starvation on the hepatotoxicity of carbon tetrachloride. Amer. Jour. Pathol. 49: 239-246.

Lehman, K.B. and Hasegawa. 1910. Studies of the absorption of chlorinated hydrocarbons in animals and humans. Arch. Hyg. 72: 327-342. (Ger.)

Mansuy, D., P. Beaune, T. Cresteil, M. Lange and J.P. Lerous. 1977. Evidence for phosgene formation during liver microsomal oxidation of chloroform. Biochem. Biophys. Res. Commun. 79: 513-517.

McConnell, G., D.M. Ferguson and C.R. Pearson. 1975. Chlorinated hydrocarbons and the environment. Endeavor. 34: 13-18.

McLean, A.E. 1970. The effects of protein deficiency and microsomal enzyme induction by DDT and phenobarbitone on the active toxicity of chloroform. Brit. Jour. Exp. Pathol. 51: 317-321.

Miklashevskii, V.E., V.N. Tugarinova, N.L. Rakhmanina and G.P. Yakovleva. 1966. Toxicity of chloroform administered perorally. Hyg. Sanit. 31: 320-322.

Morris, L.E.V. 1951. Chloroform in blood and respired atmosphere. In: Chloroform, A Study After 100 Years. R.M. Waters, ed., Madison, University of Wisconsin Press. pp. 95-119.

Munson, E.S. 1973. Discussion: Chloroform, halothane and regional anesthesia. Anesth. Analg. 52: 8-9.

Murray, F.J., B.A. Schwetz, J.G. McBride and R.E. Staples. 1979. Toxicity of inhaled chloroform in pregnant mice and their offspring. Toxicol. Appl. Pharmacol. 50: 515-522.

Nakajima T. and A. Sato. 1979. Enhanced activity of liver drug-metabolizing enzymes for aromatic and chlorinated hydrocarbons following food deprivation. Toxicol. Appl. Pharmacol. 50: 549-556.

New, P.S., G.D. Lubash, L. Scherr and A.L. Rubin. 1962. Acute renal failure associated with carbon tetrachloride intoxication. Jour. Amer. Med. Assoc. 181: 903-906.

NIOSH (National Institute for Occupational Safety). 1977b. National Occupational Hazard Survey. Vol. III. Survey Analyses and Supplemental Tables. Cincinnati, USDHEW. pp. 2864-2866.

OSHA (U.S. Occupational Safety and Health Administration). 1978. Air contaminants. U.S. Code Fed. Regl., Title 29, part 1910" 1000. pp. 612-614.

Paul, G.G. and D. Rubinstein. 1963. Metabolism of carbon tetrachloride and chloroform by the rat. Jour. Pharmacol. Exp. Ther. 141: 141-148.

Pawlowsky, R. and M.F. Frosolono. 1977. Effect of phosgene on rat lungs after single high exposure. Arch. Environm. Health. 32: 278-283.

Pegg, A.E. and G. Hui. 1978. Formation and subsequent removal of O^6-methylguanine from deoxyribonucleic acid in rat liver and kidney after small doses of dimethylnitrosamine. Biochem. J. 173: 739-748.

Piersol, G.M., H.J. Tiemen and L.S. Kau. 1933. Fatal poisoning following the ingestion of chloroform. Med. Clin. North Amer. 17: 587-601.

Plaa, G.L. and R.E. Larson. 1965. Relative nephrotoxic properties of chlorinated methane, ethane, and D24 ethylene derivatives in mice. Toxicol. Appl. Pharmacol. 7: 37-44.

Plaa, G.L., E.A. Evans and C.H. Hine. 1958. Relative hepatotoxicity of seven halogenated hydrocarbons. Jour. Pharmacol. Exp. Ther. 123: 224-229.

Pohl, L.R. 1980. Investigations of the mechanism of activation of chloroform. Toxicol. Res. Proj. Director. 5: No. 8.0463.

Pohl, L.R., B. Bhooshan, N.F. Whitaker and G. Krishna. 1977. Phosgene: A metabolite of chloroform. Biochem. Biophys. Res. Commun. 79: 684-691.

Pohl, L.R. and G. Krishna. 1978. Deuterium isotope effect in bioactivation and hepatotoxicity of chloroform. Life Science 23: 1067.

Poyer, J.R., R.A. Floyd, P.B. McCoy, E.G. Janzen and E.R. Davis. 1978. Spin-trapping of the trichloromethyl radical produced during enzymic NADPH oxidation in the presence of carbon tetrachloride or bromotrichloroethane. Biochem. Biophys. Acta. 539: 402-409.

Recknagel, R.O. and A.K. Ghoshal. 1966. Quantitative estimation of peroxidative degeneration of rat liver microsomal and mitochondrial lipids after carbon tetrachloride poisoning. Exp. Molec. Pathol. 5: 413-426.

Rechnagel, R.O. and E.A. Glende, Jr. 1973. Carbon tetrachloride hepatotoxicity: An example of lethal cleavage. CRC Crit. Rev. Toxicol. 2: 263-297.

Reitz, R.H., J.F. Quast, W.T. Scott, P.G. Watanabe and O.J. Behring. 1980. Pharmacokinetics and macromolecular effects of chloroform in rats and mice: Implications for carcinogenic risk estimation. Water Chlorinat. Environ. Impact Health Eff. 3: 983-993.

Reitz, R.H., P.J. Gehring and C.N. Park. 1978. Carcinogenic risk estimation for chloroform: An alternative to EPA's procedures. Food Cosmet. Toxicol. 16: 511-514.

Reynolds, E.S. 1967. Liver parenchymal cell injury. IV. Pattern of incorporaton of carbon and chlorine from carbon tetrachloride into chemical constituents of liver *in vivo*. Jour. Pharmacol. Exp. Ther. 155: 117-126.

Rubinstein, D. and L. Kanics. 1964. The conversion of carbon tetrachloride and chloroform to carbon dioxide by rat liver homogenates. Can. Jour. Biochem. 42: 1577-1585.

Sato, A. and T. Nakajima. 1979. A structure-activity relationship of some chlorinated hydrocarbons. Arch. Environm. Health. 34: 69-75.

Scholler, K.L. 1970. Modification of the effects of chloroform on the rat liver. Brit. Jour. Anaesth. 42: 603-605.

Schroeder, H.G. 1965. Acute and delayed chloroform poisoning. A case report. Brit. Jour. Anaesth. 37: 972-975.

Schwenkenbecher. 1904. The absorption capability of the skin. Arch. Anat. Physiol. pp. 121-165.

Schwetz, B.A., B.K.J. Leong and P.J. Gehring. 1974. Embryo- and fetotoxicity of inhaled chloroform in rats. Toxicol. Appl. Pharmacol. 28: 442-451.

Seppelt, K. 1977. Trifluromethanol, CF_3OH. Angew. Chem. Int. Ed. Engl. 16: 322-323.

Shah, H., S.P. Hartman and S. Weinhouse. 1979. Formation of carbonyl chloride in carbon tetrachloride metabolism by rat liver *in vitro*. Cancer Res. 39: 3942-3947.

Shubik, P. and A. L. Ritchie. 1953. Sensitivity of male DBA mice to the toxicity of chloroform as a laboratory hazard. Science. 117: 285.

Simpson, J.Y. 1847. On a new anaesthetic agent, more efficient than sulfuric ether. Lancet. 2: 549-550.

Sipes, I.G., G. Krishna and J.R. Gillette. 1977. Bioactivation of carbon tetrachloride, chloroform and bromotrichloromethane: Role of cytochrome P-450. Life Sci. 20: 1541-1548.

Slater, T.F. 1972. Free Radical Mechanisms in Tissue Injury. Pion. Ltd., London.

Smith, A.A., P.O. Volpetto, Z.W. Gremling, M.B. DeVore and A.B. Glassman. 1973. Chloroform, halothane and regional anesthesia. A comparative study. Anesth. Analg. 52: 1-11.

Snow, J. 1858. On chloroform and other anesthetics. London, John Churchill.

Steward, A., P.R. Allot, A.L. Cowles and W.W. Mapleson. 1973. Solubility coefficients for inhaled anaesthetics for water, oil, and biological media. Brit. Jour. Anaesth. 45: 282-293.

Stewart, R.D. 1974. The use of breath analysis in clinical toxicology. In: Essays in Toxicology. New York, Academic Press. Chapter 5.

Stewart, R.D., H.C. Dodd, D.S. Erly and B.B. Holder. 1965. Diagnosis of solvent poisoning. Jour. Amer. Med. Assoc. 193: 1097-1100.

Storms, W.W. 1973. Chloroform parties. Jour. Amer. Med. Assoc. 225: 160.

Symons J.M., T.A. Bellar, J.K. Carswell, J. DeMarco, K.L. Kropp, G.G. Robeck, D.R. Seeger, C.J. Slocum, B.L. Smith and A.A. Stevens. 1975. National Organics Reconnaissance Survey for Halogenated Organics. Amer. Water Works Assoc. Jour. 67: 634-647.

Taylor, D.C., D.M. Brown, R. Kuble and P.F. Langley. 1974. Metabolism of chloroform. II. A sex difference in the metabolism of ^{14}C-chloroform in mice. Xenobiotica. 4: 165-174.

Thompson, D.J., S.D. Warnet and V.V. Robinson. 1974. Teratology studies on orally administered chloroform in the rat and rabbit. Toxicol. Appl. Pharmacol. 29: 348-357.

Torkelson, T.R., F. Oyen and V.K. Rowe. 1976. The toxicity of chloroform as determined by single and repeated exposure of laboratory animals. Amer. Ind. Hyg. Assoc. Jour. 37: 697-704.

Tsurata, H. 1975. Percutaneous absorption of organic solent. 1. Comparative study of the in vivo percutaneous absorption of chlorinated solvents in mice. Ind. Health. 13: 227-236.

Tsurata, H. 1977. Percutaneous absorption of organic solvents. 2. A method for measuring the penetration rate of chlorinated solvents through excised rat skin. Ind. Health. 15: 131-140.

Uehleke, H. and T. Werner. 1975. A comparative study on the irreversible binding of labeled halothane, trichlorofluoromethane, chloroform, and carbon tetrachloride to hepatic protein and lipids in vitro and in vivo. Arch. Toxicol. 34: 289-303.

Uehleke, H., K.H. Hellmer and S. Tarabelli. 1973. Binding of ^{14}C-carbon tetrachloride to microsomal proteins in vitro and formation of $CHCl_3$ by reduced liver microsomes. Xenobiotic. 3: 1-11.

Uehleke, H., T. Werner, H. Greim and M. Kramer. 1977. Metabolic activation of halothanes and tests in vitro for mutagenicity. Xenobiotica. 7: 393-400.

Van Dyke, R.A., M.B. Chenoweth and A. van Poznak. 1964. Metabolism of volatile anesthetics. I. Conversion in vivo of several anesthetics to $^{14}CO_2$ and chloride. Biochem. Pharmacol. 13: 1239-1247.

Villarruel, M.C. and J.A. Castro. 1975. Irreversible binding of carbon tetrachloride to microsomal phospholipids. Free radical nature of the reactive species and alterations in the physico-chemical properties of the target fatty acids. Res. Commun. Chem. Pathol. Pharmacol. 10: 105-115.

Villarruel, M.C., M.I. Diaz-Gomes and J.A. Castro. 1975. The nature of the irreversible binding of carbon-tetrachloride to microsomal lipids. Toxicol. Appl. Pharmacol. 33: 106-116.

von Oettingen, W.F. 1964. The Halogenated Hydrocarbons of Industrial and Toxicological Importance. Amsterdam, Elsevier Publ. Co. pp. 77-108.

Waters, R.M. 1951. Chloroform. A Study After 100 Years. Madison, University of Wisconsin Press.

Withey, J.R., B.T. Collins and P.G. Collins. 1982. Effect of vehicle on the pharmacokinetics and uptake of four halogenated hydrocarbons from the gastrointestinal tract of the rat. Pre-print paper submitted to J. Appl. Toxicol., December, 1982.

Withey, J.R. and B.T. Collins. 1980. Chlorinated aliphatic hydrocarbons used in the foods industry: The comparative pharmacokinetics of methylene chloride, 1,2-dichloroethane, chloroform and trichloroethylene after i.v. administration in the rat. J. Environ. Path. Toxicol. 3: 313-332.

Witte, C. 1874. XXXIV. Experiments concerning the effects of chloroform on blood circulation. Dtsch. Z Chir. 4: 548-566.

Wolf, C.R., D. Mansuy, W. Nastainczyk, G. Deutschmann and V. Ullrich. 1977. The reduction of polyhalogenated methanes by liver microsomal cytochrome P-450. Molec. Pharmacol. 13: 698-705.

Zeller, A. 1883. On the fate of iodoforms and chloroform in the organism. Hoppe-Seyler's Z. Physiol. Chem. 8: 78-79.

5. TOXICITY

5.1 EFFECTS OF ACUTE EXPOSURE TO CHLOROFORM

In both humans and experimental animals, characteristic effects of acute exposure to chloroform are depression of the central nervous system and hepatic damage. Renal and cardiac effects also occur. The systemic toxic effects of chloroform appear to be similar regardless of whether exposure or administration occurred by inhalation, oral, or parenteral routes. The only systemic effect documented for dermal administration, however, is renal damage.

5.1.1 Humans

5.1.1.1 ACUTE INHALATION EXPOSURE IN HUMANS -- Information on the effects of acute inhalation exposure of chloroform on humans has been obtained primarily during its use as an inhalation anesthetic. The relationship of the concentration of chloroform in inspired air and blood to anesthesia is described in Table 5-1 (Goodman and Gilman, 1980). Concentrations of chloroform used for the induction of anesthesia were in the range of 2-3 volumes % (20,000-40,000 ppm), followed by lower maintenance levels (NIOSH, 1974; Adriani, 1970).

Chloroform inhalation has a depressive effect on the central nervous system. Excitement due to release of inhibitions is followed by progressive depression of the cortex, higher centers, medulla and spinal cord (Wood-Smith and Stewart, 1964). Centers controlling temperature regulation, respiration, vomiting, vasomotor, and vagal activity are all depressed (Adriani, 1970).

The cardiovascular system is also affected by anesthetic use of chloroform. The myocardium is directly depressed in deeper planes of anesthesia. A blood level sufficient to cause respiratory failure may also cause cardiac arrest. In addition, chloroform sensitizes the autonomic tissues of the heart to epinephrine, causing arrhythmias. It has been found that under chloroform

TABLE 5-1

Relationship of Chloroform Concentration in Inspired Air and Blood to Anesthesia*

	In Inhaled Air Volumes %	In Blood mg%
Not sufficient for anesthesia	<0.15	<2
Light anesthesia (after induction)	0.15 to 0.20	2 to 10
Deep anesthesia	0.20 to 1.50	10 to 20
Respiratory failure	2.0	20 to 25

*Source: Goodman and Gilman, 1980

anesthesia regarded as normal, the heart is subject to arrythmias and extrasystoles (Kurtz et al., 1936; Orth et al., 1951). Orth et al. (1951) found a high incidence of ventricular arrhythmias, 20 of 52 cases investigated, and four cases of temporary cardiac arrest. Blood pressure is lowered by chloroform as a result of a 3-fold action: cardiac slowing due to vagal stimulation, depression of the vasomotor center, and dilation of splanchnic blood vessels (Krantz and Carr, 1965).

Respiratory effects of chloroform inhalation include increased rate and depth of respiration during induction and in light anesthesia, and decreased minute volume exchange in deeper planes of anesthesia. The Hering-Breuer reflex remains active. Bronchial smooth muscle is relaxed and secretions are increased. Laryngeal spasms are caused by high concentrations (Adriani, 1970).

In the gastrointestinal tract, chloroform markedly stimulates the flow of saliva during induction and recovery, but salivation is inhibited in deeper planes of anesthesia (Goodman and Gilman, 1980). The pharyngeal or gag reflex is depressed. Under anoxic conditions, pharyngeal muscle spasms result in stertorous respiration and thick mucus is excreted (Adriani, 1970). Stomach movements are decreased or abolished as tone is reduced. Gastric secretory activity is inhibited or abolished. Post-anesthetic dilation of the stomach occurs in nearly all cases. Nausea and vomiting often occur during recovery from anesthesia. The mechanism is central rather than local, but may be due in part to irritation of the stomach by swallowed vapor (Goodman and Gilman, 1980). Intestinal tone, motility, and secretory activity are inhibited or abolished (Adriani, 1970).

In the urinary tract, chloroform anesthesia results in a decrease in urine flow, possibly due to the release of antidiuretic hormone and renal vasoconstriction, leading to a decrease in renal blood flow and glomerular filtration.

Polyuria occurs after recovery (Goodman and Gilman, 1980). Chloroform anesthesia may be followed by albuminuria and glycosuria. Post-operative urine retention occurs frequently. Renal tubular necrosis has been found in cases of severe poisoning (Wood-Smith and Stewart, 1964).

During obstetric use of chloroform, uterine contractions are only slightly decreased by light anesthesia, but are markedly inhibited in deeper planes. Chloroform rapidly crosses the placental barrier, and respiratory depression in the infant is likely to occur (Wood-Smith and Stewart, 1964).

Chloroform anesthesia also has metabolic effects in humans. A rise in blood glucose accompanies anesthesia. Levels may rise $\geq$2-fold and remain elevated for several hours. The liver glycogen falls coincident with the rise in blood sugar. This, in turn, is a result of the release of epinephrine from the adrenal medulla during the period of excitation. There is also a decrease in glucose utilization in the periphery (Goodman and Gilman, 1980; Krantz and Carr, 1965). Acidosis occurs, characterized by a fall in plasma biocarbonate and phosphate.

Chloroform is acutely toxic to the liver, although in so-called delayed chloroform poisoning, the full effects of damage done during and shortly after administration are not seen for 24-48 hours. The glycogen content of the liver is rapidly depleted; three-fourths in the first half-hour and less rapidly thereafter. There is centrilobular and, in severe cases, mid-zonal and massive necrosis. Cells which survive show fatty degeneration. Symptoms include progressive weakness, prolonged vomiting, delirium, coma, and death. They develop from the first to the third day after exposure. Jaundice, increased serum bilirubin, bile in the urine, reduction in liver function, increased nitrogen excretion, lowered blood prothrombin and fibrinogen, and the appearance of leucine, tryosine, acetone, and diacetic acid in the urine are some of the more prominent findings. The hemorrhagic tendency is due to reduced prothrombin formation by the injured liver. Death usually occurs on the fourth or fifth day, and autopsy reveals degeneration and necrosis of liver tissue, most marked around the central veins (Goodman and Gilman, 1980; Wood-Smith and Stewart, 1964).

Hematologic effects due to acute chloroform inhalation are seen during anesthesia. Erythrocytes are increased in number as the spleen is constricted and red blood cells are extruded into the circulation. Leukocytes are increased in number during the post-anesthetic period, reaching a maximum within 24 hours and returning to normal in 48 hours. There is an increase in polymorphonuclear cells. Platelets remain unchanged. One half-hour after exposure, there is a decrease in clotting time. Prothrombin time is increased. Prothrombin synthesis is impaired by liver toxicity as previously noted (Adriani, 1970).

The effects of chloroform on the eye include dilation of the pupils, with reduced reaction to light as well as reduced intraocular pressure (Sax, 1979; Winslow and Gerstner, 1978).

Signs of chloroform poisoning include a characteristic sweetish odor on the breath, cold and clammy skin, and dilated pupils (Winslow and Gerstner, 1978). Nausea and vomiting commonly occur. Ketosis, due to incomplete oxidation of fats, as well as a rise in blood sugar, accompanies chloroform intoxication. Initial excitation alternating with apathy is followed by prostration, unconsciousness, and possible death due to cardiac and central nervous system depression (Winslow and Gerstner, 1978).

The above discussion presents observations made on the effects of chloroform inhalation during general anesthesia. Information on the effects of experimental acute inhalation exposure of chloroform in humans is limited to the work of Lehman and Hasegawa (1910) and Lehman and Schmidt-Kehl (1936) as reviewed by NIOSH (1974). The duration of exposure was $\leq$30 minutes and only the subjective responses of the subjects were measured. The dose-response relationships as tabulated by NIOSH (1974) are presented in Table 5-2.

5.1.1.2 ACUTE ORAL EXPOSURE IN HUMANS -- Case reports of suicides (Piersol et al., 1933; Schroeder, 1965) and of recreational abuse (Storms, 1973) of chloroform present some information on the effects of acute imbibition. A fatal dose of ingested chloroform may be as little as one-third of an ounce (10 mℓ) (Schroeder, 1965). The initial effect is usually unconsciousness and possibly death (within 12 hours without treatment) due to respiratory or cardiac arrest. If the patient survives, delayed effects are observed within 48 hours after regained consciousness. These symptoms include vomiting, anorexia, jaundice, liver enlargement, albuminuria, ketosis, ketonuria and glucosuria, hemorrhage due to lowered blood fibrinogen and prothrombin, reduced serum bicarbonate, increased blood sugar, coma and possible death. Upon autopsy, extensive hepatic centrilobular necrosis is evident.

5.1.1.3 ACUTE DERMAL AND OCULAR EXPOSURE IN HUMANS -- Chloroform is absorbed through the intact skin (von Oettingen, 1964). Application of chloroform to the skin is followed after 3 minutes by a pungent and burning pain reaching its maximum after 5 minutes, associated with erythema, hyperemia, and finally vesication (Oettel, 1936). Exposure of the eye to concentrated chloroform vapors causes a stinging sensation. Splashing the substance into the eyes evokes burning, pain, and redness of the conjunctival tissue. The corneal epithelium is sometimes impaired; however, regeneration starts rapidly and leads to full recovery within 1-3 days (Winslow and Gerstner, 1978).

5.1.2 Experimental Animals

5.1.2.1 ACUTE INHALATION EXPOSURE IN ANIMALS -- Tolerance of animals to chloroform has been summarized by Lehmann and Flury (1943) and by Sax (1979). Similar central nervous system effects are seen in animals at approximately the

TABLE 5-2

Dose-Response Relationships*

160 ppm (0.8 mg/ℓ) for unspecified time - no odor

205 ppm (1.0 mg/ℓ) for unspecified time - light transient odor

390 ppm (1.9 mg/ℓ) for 30 minutes -light transient odor

920 ppm (4.5 mg/ℓ) for 7 minutes - stronger, lasting odor; dizziness, vertigo after 3 minutes

680 ppm (3.3 mg/ℓ) to 1000 ppm (5.0 mg/ℓ) for 30 minutes - moderately strong odor; taste

1100 ppm (5.4 mg/ℓ) for 5 minutes - still stronger, permanent odor; dizziness, vertigo after 2 minutes

1400 ppm (6.6 mg/ℓ) to 1800 ppm (8.57 mg/ℓ) for 30 minutes - stronger odor, tiredness, salivation, giddiness, vertigo, headache, taste

3000 ppm (14.46 mg/ℓ) for 30 minutes - all above plus pounding heart, gagging

4300 ppm (20.8 mg/ℓ) to 5000 ppm (25 mg/ℓ) for 20 minutes - dizziness and light intoxication

5100 ppm (25 mg/ℓ) for 20 minutes - dizziness and light intoxication

7200 ppm (35.3 mg/ℓ) for 15 minutes - dizziness and light intoxication as above but more pronounced same magnitude of exposure that produced these effects in humans. In mice, exposure to 2500 ppm for 2 hours produced no obvious effects, 3100 ppm for 1 hour produced slight narcosis, while 4000 ppm induced deep narcosis within one-half hour. Only slight symptoms are seen at 2000-6000 ppm for longer exposures. Fatal exposures were 4100-8200 ppm for mice, 12,300 ppm for rabbits, and 16,300-20,500 ppm for guinea pigs (duration of fatal exposures not specified). In cats, exposure to 7200 ppm resulted in disturbance of the equilibrium after 5 minutes, light narcosis after 60 minutes, and deep narcosis after 93 minutes of exposure. Exposure to 21,500 ppm produced disturbances in equilibrium after 5 minutes, light narcosis after 10 minutes, and deep narcosis in cats after 13 minutes of exposure.

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*Source: Lehman and Hasegawa (1910) and Lehman and Schmidt-Kehl (1936).

Kylin et al. (1963) described the effects of a single exposure of mice to 100, 200, 400, or 800 ppm of chloroform for 4 hours. The mice exposed to 100 ppm did not develop demonstrable liver necroses, although moderate fatty infiltration of the liver was noted. In mice exposed to 200 ppm, some necrotic areas appeared in the liver and there was an increase in serum ornithine-carbamyl transferase. Exposure to chloroform at 400 and 800 ppm resulted in increased hepatic necrosis and serum enzyme activity.

More recent data regarding toxic effects of acute inhalation exposure to chloroform were presented by Wood et al. (1982), although the study was designed primarily to investigate the role of hydrogen bonding in the anesthetic mechanism. Groups of mice in a rotating cage were given a single exposure of upto 3 hours of varying concentrations of chloroform or deuterated chloroform, each concentration being held constant for about 20 minutes and then being raised until the mice had lost their righting reflex. The concentration was then lowered to 1/2 the ED_{50} (≈1500 ppm) where it remained until the mice had regained their righting reflex. The duration of these manipulated exposures never exceeded 3 hours. Only 4 of 47 mice given chloroform gained the righting reflex; indeed, some mice died or were comatose. Upon histological examination of animals sacrificed 3-6 hours after exposure, mild hepatic centrilobular necrosis and very mild renal tubular necrosis was observed. The animals receiving deuterated chloroform survived for 24 hours, after which they were sacrificed. The liver and kidney lesions in these mice were more severe, perhaps owing to the longer survival time, which may have allowed these lesions to develop.

5.1.2.2 ACUTE ORAL EXPOSURE IN ANIMALS

Kimura et al. (1971) performed acute oral toxicity studies in newborn (5-8 g), 14-day-old (16-50 g), young adult (80-160 g), and older adult (360-470 g) rats. The chloroform was given in undiluted form to unfasted rats. LD_{50} values in mℓ/kg (95% confidence limits) were reported as follows: 14-day-old, 0.3 (0.2-0.5); young adult, 0.9 (0.8-1.1), and older adult, 0.8 (0.7-0.9). The young and older adult rats were males; the other two groups contained rats of both sexes. When compared with 15 other solvents included in this study, the LD_{50} values for chloroform were the lowest in the two adult groups and next to lowest in the 14-day-old rats. Only a rough approximation of the LD_{50} could be obtained for the newborn rats; volumes of 0.01 mℓ/10 kg body weight were generally fatal. Lower volumes could not be measured with any degree of accuracy and were not attempted.

Torkelson et al. (1976) reported an oral LD_{50} of 2.0 g/kg (1.05-3.80) in male rats. Animals receiving as little as 0.25 g/kg showed adverse effects. Other recent studies of acute oral toxicity have reported LD_{50} values (with 95% confidence limits) of 1120 mg/kg (789-1590) in ICR male mice and 1400 mg/kg (1120-1680) in females (Bowman et al., 1978), and 908 mg/kg (750-1082) and 1117 mg/kg (843-1514), respectively, in male and female rats (Chu et al., 1980).

In a study that compared the toxicities of halogenated hydrocarbons, a single oral dose of 60 mg/kg chloroform to mice had no toxic effect (Hjelle et al., 1982).

Hill (1978) performed experiments in mice designed to study variability in susceptibility to chloroform toxicity from single oral doses based upon genetic sex differences. For three strains of mice, the LD_{50} values (mℓ/kg) were: DBA/2J, 0.08; B6D2F1/J, 0.20; and C57BL/6J, 0.33. The animals more sensitive to chloroform-induced death were also found to be more susceptible to renal toxicity. Males were found to be more sensitive to renal damage and death than were females. This difference was related to testosterone and it was further noted that C57BL/10J males are relatively testosterone deficient in comparison to DBA males. The C57BL/6J strain used in the Hill (1978) study is closely related to the C57BL/10J strain and may, therefore, also be testosterone deficient.

In male B6C3F1 mice, severe diffuse renal necrosis occurred after a single oral dose of 240 mg/kg and focal tubular regeneration occurred after a single dose of 60 or 240 mg/kg. These effects were not seen after 15 mg/kg (Reitz et al., 1980). Liver damage (hepatocellular necrosis and swelling with inflammatory cell infiltration) occurred only at the highest doses.

Chu et al. (1982a) studied the effects of acute oral exposure of chloroform on Sprague-Dawley rats. Groups consisted of 10 male and 10 female animals given a single oral dose of 0. 546, 765, 1071, 1500, or 2100 mg/kg of chloroform in a volume of 5 mℓ/kg corn oil. Clinical signs of toxicity included depression and coma, but the authors did not specify whether these signs occurred at all dose levels. Treated rats surviving for 14 days consumed less food and had depressed growth rates. Gross examination revealed increased liver and kidney weights at 1071 mg/kg. Upon comprehensive histological examination, only mild to moderate lesions, even at high doses, were observed in these organs. No changes were noted in other organs, including brain and heart. The hepatic and renal lesions were characterized by hepatocyte variations and occasional vesiculation of biliary epithelial nuclei in the liver, and by bilateral focal interstitial nephritis and fibrosis in the kidney. Changes in hematological and biochemical parameters were also observed in the 1071 and 1500 mg/kg treated groups. Cholesterol levels increased while lactate dehydrogenase activity and liver protein levels decreased. In female rats, the activity of microsomal aniline hydroxylase was induced by chloroform exposure. The numbers of lymphocytes were reduced in both males and females, as were hemoglobin and hematocrit values. Upon longer exposures of 5, 50, or 500 ppm chloroform in drinking water for 28 days, the only toxic effect observed was a decreased number of neutrophils in the highest dose-exposed rats. Examinations were performed as in the single-dose experiment.

5.1.2.3 ACUTE DERMAL AND OCULAR EXPOSURE IN ANIMALS -- Torkelson et al. (1976) found that chloroform, when applied to the skin of rabbits, produced slight to moderate irritation and delayed healing of abraded skin. When applied to the uncovered ear of rabbits, slight hyperemia and exfoliation occurred after one to four treatments. No greater injury was noted after 10 applications. One to two 24-hour applications, on a cotton pad bandaged on the shaven belly of the same rabbits, produced a slight hyperemia with moderate necrosis and a resulting eschar formation. Healing appeared to be delayed on the site as well as on abraded areas which were also covered for 24 hours with a cotton pad soaked in chloroform.

Single application of either 1.0, 2.0, or 3.98 g/kg for 24 hours under an impermeable plastic cuff held tightly around the clipped bellies of each of two rabbits did not result in any deaths. However, extensive necrosis of the skin and considerable weight loss occurred at all levels. All animals were sacrificed for study 2 weeks after exposure. All treated rabbits exhibited degenerative changes in the kidney tubules graded in intensity with dosage levels. The livers were not grossly affected.

In the same study (Torkelson et al., 1976), liquid chloroform, dropped into the eyes of three rabbits, caused slight irritation of the conjunctiva which was barely detectable 1 week after treatment. In addition, slight but definite corneal injury occurred, as evidenced by staining with fluorescein. A purulent exudate occurred for ≥ 2 days after treatment. Although started 30 seconds after instilling the chloroform, thorough washing of one eye of each rabbit with a stream of running water for 2 minutes did not significantly alter the response in the washed eyes from that of the unwashed eyes.

5.1.2.4 INTRAPERITONEAL AND SUBCUTANEOUS ADMINISTRATION IN ANIMALS -- The toxicity of chloroform in mice after subcutaneous administration (Kutob and Plaa, 1962b) and intraperitoneal administration (Klaassen and Plaa, 1966) has been compared with that of other halogenated hydrocarbons (Pohl, 1979). In these studies, the LD_{50} values for carbon tetrachloride, chloroform, and dichloromethane were 200, 27.5, and 76 mmol/kg after subcutaneous administration and 20, 14, and 23 mmol/kg when given intraperitoneally. When the relative hepatotoxicity of these compounds was compared, a subcutaneous dose of 0.5 mmol/kg of carbon tetrachloride produced approximately the same degree of liver damage as 6.2 mmol/kg of chloroform. After intraperitoneal administration, these values dropped to 0.01 mmol/kg for carbon tetrachloride and 2.3 mmol/kg for chloroform. Dichloromethane did not cause significant histological changes in the liver by either route of administration. At doses that produced liver toxicity, chloroform caused kidney lesions which ranged from the presence of hyaline droplets, nuclear pycnosis, hydropic degeneration, and increased eosinophilia, to necrosis with karyolysis and loss of epithelium of the convoluted tubules.

Ilett et al. (1973) found that intraperitoneal administration of chloroform caused centrilobular hepatic necrosis in mice of both sexes, whereas renal necrosis was observed only in male mice.

5.2. EFFECTS OF CHRONIC EXPOSURE TO CHLOROFORM

A characteristic effect of chronic exposure to chloroform is hepatic damage; this effect has been documented primarily in studies with experimental animals. As was the case for acute exposure to chloroform, hepatic damage in chronic studies results from either inhalation or oral administration of this chemical. Effects on the kidneys and thyroids have also been observed in some experiments. This section will discuss both subchronic (≈90 days) and chronic exposure studies, because many of the subchronic studies were preliminary range-finding tests for the chronic studies.

5.2.1. Humans

5.2.1.1 CHRONIC INHALATION EXPOSURE IN HUMANS -- Only two chronic inhalation studies that reported measurements of exposure concentrations, as well as effects on human health, were found. Neither study (Challen et al., 1958; Bomski et al., 1967) is particularly adequate or recent.

Challen et al. (1958) investigated complaints of workers (mainly women) in a plant manufacturing lozenges that contained chloroform as a principle ingredient. Before exhaust ventilation was installed, 9 of the 10 exposed workers had complained of symptoms of tiredness, dull-wittedness, depression, gastrointestinal distress, and frequent and scalding urination. Breathing-zone monitoring during simulation of "pre-ventilation" working conditions suggested that the emplyees had been exposed to ≈77-237 ppm. Discussions with management revealed that some of these workers had occasionally been observed to behave in a silly manner or to stagger about during the workday. Another group of workers (N = 10) had been exposed primarily to concentrations of 22-71 ppm. Eight of these workers complained of less severe symptoms. Apparently, both groups of workers had been exposed to occasional peak concentrations of ≈1163 ppm lasting 1.5-2 minutes. At least four workers in each group worked half-time. None of the five controls reported symptoms similar to those reported by the exposed workers. Eight of the higher exposure employees (77-237 ppm for 3-10 years, followed by ≈2 years without exposure), nine of the lower exposure employees (22-77 ppm for 10-24 months), and five unexposed employees submitted to physical examinations, including liver function tests (thymol turbidity, serum bilirubin, and urine urobilinogen tests). These examinations and tests revealed no evidence of any organic lesion, including liver damage, attributable to exposure to chloroform.

In humans, hepatic damage is the most common toxic effect of acute exposure to chloroform, as noted previously. According to Pohl (1979), only one report of liver abnormalities in humans after chronic exposure to chloroform has been found in the literature and no additional reports were found in the more recent literature. In this study (Bomski et al., 1967), 17 cases of hepatomegaly were found in a group of 68 industrial workers who were exposed to chloroform in concentrations ranging from 2-205 ppm for 1-4 years. These were unknown rather than breathing zone concentrations. Three of the 17 workers with hepatomegaly were judged to have toxic hepatitis on the basis of elevated serum enzymes. The frequency of viral hepatitis among the 68 chloroform-exposed workers was higher (4.4% versus 0.38%) than the frequency among a group of inhabitants of the city, ≥18 years of age. This phenomenon also occurred in the 2 previous years. Ten cases of splenomegaly were also diagnosed among the 68 workers. There appears to be no comparison with incidences of these conditions in nonexposed workers.

5.2.1.2 CHRONIC ORAL EXPOSURE IN HUMANS -- There are relatively few reports of toxic effects following chronic ingestion of chloroform (Pohl, 1979), and more recent reports were not located in the literature. In one case, it was estimated that a male patient ingested 1.6-2.6 g of chloroform in a cough medicine daily for ≈10 years (Wallace, 1950). Blood and urine analyses, as well as liver function tests, indicated the individual suffered from hepatitis and nephrosis. Another report described three patients addicted to chlorodyne, a tincture containing chloroform and morphine (Conlon, 1963). Liver biopsy showed severe cellular damage in one of these individuals who had ingested 21 mℓ of chloroform daily for an undetermined period of time. All three displayed evidence of serious mental and physical deterioration, including peripheral neuropathy. It is not possible to determine if the adverse effects were due to chloroform, morphine, or ethanol.

More recently, the safety of a dentifrice containing 3.4% chloroform and a mouthwash containing 0.43% was assessed in studies lasting ≥1 year (DeSalva et al., 1975). The subjects using the dentifrice were exposed to ≈70 mg (0.047 mℓ) of chloroform each day, whereas the groups using the mouthwash were exposed to ≈178 mg (0.12 mℓ). The results of liver function tests and blood urea nitrogen determinations showed no statistical differences between control and experimental subjects.

Epidemiologic studies of humans exposed to chloroform in their drinking water have focused on carcinogenic endpoints, and, hence, are discussed in the chapter on carcinogenicity.

5.2.2 Experimental Animals

5.2.2.1 CHRONIC INHALATION EXPOSURE IN ANIMALS -- Experiments with several species of animals (Torkelson et al., 1976) give some information regarding potential effects of long-term inhalation exposure to chloroform. The animals were exposed to chloroform 5 days/week for 6 months. Exposure to 25 ppm of chloroform for up to 4 hours/day had no adverse effects in male rats as judged by organ and body weights, and by gross and histological examination of livers and kidneys. Exposure to 25 ppm for 7 hours/day, however, produced histopathological changes in the livers and kidneys of male but not female rats. These changes were characterized as lobular granular degeneration and focal necrosis throughout the liver and cloudy swelling of the kidneys. The hepatic and renal effects appeared to be reversible because rats exposed according to the same protocol, but given a 6 week recovery period following exposure, appeared normal by the criteria tested. Increasingly pronounced changes were observed in the livers and kidneys of both sexes of rats exposed to 50 or 85 ppm for 7 hours/day. Hematologic indices, clinical chemistry, and urinalysis values, tested at higher levels of exposure, were within normal limits. Each exposure group and control group had 10-12 rats/sex except for the 25 ppm, 4 hour/day group, which had 10 male rats and no females.

Similar experiments with guinea pigs (N = 8-12 sex/group) and rabbits (N = 2-3/sex/group) gave somewhat inconsistent results. Histopathological changes were observed in livers and kidneys of both species at 25 ppm but not at 50 ppm in either species, nor even at 85 ppm in guinea pigs. The results of these studies are summarized in Table 5-3.

Other reports of effects of chronic inhalation exposure to chloroform in experimental animals were not found in the more recent literature.

5.2.2.2 CHRONIC ORAL EXPOSURE IN ANIMALS -- The data from several studies on the effects of chronic and subchronic oral exposure to chloroform are summarized in Table 5-4. Low levels of exposure (15-64 mg/kg/day, 6 days/week) have been reported to increase survival in mice and rats (Roe et al., 1979; Palmer et al., 1979) and to be associated with possible transient CNS depression

TABLE 5-3

Effects of Inhalation Exposure of Animals to Chloroform, 5 Days/Week for 6 Months*

Species	Sex	ppm	Exposure hours/day	Number in Group Started	Number in Group Survived	Effects
rats	M	85	7	10	6	Excess mortality attributed to pneumonia on basis of gross and microscopic appearance of lungs; slight depression of final body weight; increase ($p<0.05$) in relative but not absolute weights of liver, kidneys, and testes; no effect on spleen weight; histological findings included marked centrilobular granular degeneration of the livers and cloudy swelling of the kidneys but no histopathological changes in testes; hematologic values (including differential count), urinalysis values and SGPT, SUN, and SAP values all "within normal limits"
	F	85	7	10	10	No evidence of pneumonia; final body weights and weights of liver and spleen unaffected, relative and absolute kidney weights increased; histological findings included marked centrilobular granular degeneration of the livers and cloudy swelling of the kidneys; hematologic, urinalysis, and SGPT, SUN, and SAT values all "within normal limits"
	M	50	7	10	9	Depression of final body weights ($p<0.05$); increases in relative ($p<0.05$) but not absolute weights of kidneys, spleen, and testes; histopathological changes in livers and kidneys similar to those seen at 85 ppm; hematologic values, urinalysis values, and SGPT, SUN, and SAP values all "within normal limits"
	F	50	7	10	10	Final body weights and weights of liver and spleen unaffected; increase in relative kidney weight ($p <0.05$); histopathological changes in livers and kidneys similar to those seen at 85 ppm but somewhat less marked; hematologic values, urinalysis values, and SGPT, SUN, and SAP values all "within normal limits"
	M	25	7	12	9	No effect on final body weights or weight of liver, spleen, or testes; increased relative kidney weight ($p <0.05$); lobular granular degeneration with focal areas of necrosis throughout the liver; cloudy swelling of renal tubular epithelium
	F	25	7	12	12	No statistically significant effect on body weight; increased relative but not absolute kidney and spleen weights; no histopathologic changes in kidneys and spleen; microscopic appearance of livers not specified

TABLE 5-3

Effects of Inhalation Exposure of Animals to Chloroform, 5 Days/Week for 6 Months* (cont.)

Species	Sex	ppm	Exposure hours/day	Number in Group Started	Number in Group Survived	Effects
rats	M,F	25 plus 0 ppm for 6 weeks (recovery period)	7	12/sex	8 M, 10 F	"Normal" by the criteria tested at this dosage level (see 25 ppm above)
	M	25	1,2, or 4	10, 10, 10	7,8,4, respectively	No evidence of adverse effects by the criteria tested (i.e., final body weight; weights of livers, kidneys, spleen, testes; and probably gross and microscopic appearance of at least the liver and kidneys)
guinea pigs	M,F	85,50, or 25	7	8 to 12/sex/ exposure level	50 to 92% (mortality not related to exposure)	No adverse effects at 50 or 85 ppm other than marked pneumonitis in F at 85 ppm; some histopathological changes in livers of both sexes and kidneys of M at 25 ppm (criteria tested were body weights, organ weights, and gross and microscopic appearance of organs)
rabbits	M,F	85, 50, or 25	7	2 to 3/sex/ exposure level	0 to 1 death/ group, not related to exposure	No adverse effects at 50 ppm; some histopathologic changes in kidneys and liver and pneumonitis in lungs at 25 and 85 ppm. Hematologic and clinical chemistry values within normal limits at 85 ppm (criteria tested were same as for rats)
dogs	M,F	25	7	1/sex	1/sex	No adverse effects in M; marked cloudy swelling of renal tubular epithelium and increase in capsular space in glomeruli of kidneys in F (criteria tested were same as for rats and included clinical chemistry and hematological studies)

*Source: Torkelson et al., 1976

M = male; F = female; SGPT = serum glutamic pyruvic transaminase; SUN = serum urea nitrogen; SAP = serum alkaline phosphatase

Controls for each species and sex included at least one unexposed and one air-exposed group, each comparable in number of animals to the exposed groups. In statistical comparisons of organ and body weights, values for the control group (unexposed or air-exposed) closer in body weight to the test group were used. Mortality in control groups was similar to mortality in treated groups, with the exception of excess mortality in male rats exposed to 85 ppm for 7 hours/day or to 25 ppm for 4 hours/day. No explanation was given by Torkelson et al. (1976) for the high mortality in the 4 hours/day group. Strains of animals and age or weight at the start of the experiment were not specified. Purity of the chloroform used was 99.3% (0.4% ethyl alcohol and <0.3% of an unknown).

TABLE 5-4

Effects of Subchronic or Chronic Oral Administration of Chloroform to Animals

Species, Strain Age/Weight at Start	Sex	No. at Start	Vehicle	Dosage	Duration	Response	Reference
Rats, Sprague-Dawley weanling, 101 g M, 94 g F	M,F	20/sex/dose level	drinking water	0, 5, 50, 500, or 2500 ppm in drinking water ad lib. corresponding to intakes^c of 0, 0.11-0.71, 1.2-1.5, 8.9-14, or 29-55 mg/rat/day The highest dose^c corresponds to ≈291 mg/kg/day F, 310 mg/kg/day M	90 days, after which 10 rats/group were killed and 10 rats/group observed for additional 90 days	Increased mortality, decreased growth rate, and decreased food intake at highest dose; increased frequency of mild to moderate liver and thyroid lesions at highest dose, including increases in cytoplasmic homogeneity, hepatocyte density, and cytoplasmic volume, vacuolization due to fatty infiltration, some vesiculation of biliary epithelial nuclei, and hyperplasia in livers; and reduced follicle and colloid density, increased epithelial height, some focal collapse of follicles in thyroid; no histopathological effects in kidney, brain, and heart; after the 90-day recovery, the lesions were very mild and similar to those seen in controls	Chu et al., 1982b

TABLE 5-4

Effects of Subchronic or Chronic Oral Administration of Chloroform to Animals (cont.)

Species, Strain Age/Weight at Start	Sex	No. at Start	Vehicle	Dosage	Duration	Response	Reference
Rats, Osborne-Mendel 6 weeks/190 g	M	30/group except 40 ad lib. controls	drinking water	0, 200, 400, 600 900, or 1800 ppm in drinking water ad lib. corresponding to intakes[a] of 0, 20, 38, 57, 81, or 160 mg/kg/day, plus 0 ppm group matched with 1800 ppm group for water consumption	10 rats/group killed at 30, 60, and 90 days of exposure	Dose-related signs of depression during 1st week only; dose-related reduction in water consumption; decreased weight gain in 160 mg/kg group; increased incidence of "hepatosis" in livers of treated rats at 30 and 60 days but not 90 days (not dose-related); no other treatment-related effects on serum clinical chemistry values or urinalysis values, or gross and microscopic appearance of tissues, including kidney.	Jorgenson and Rushbrook, 1980

TABLE 5-4

Effects of Subchronic or Chronic Oral Administration of Chloroform to Animals (cont.)

Species, Strain Age/Weight at Start	Sex	No. at Start	Vehicle	Dosage	Duration	Response	Reference
rats, Osborne-Mendel 52 days/240 g M 175 g F	M,F	50/sex/dose level; 20/sex matched controls; ≈99/sex colony controls	corn oil, gavage	M: 0, 90, or 180 mg/kg/day; F: 0, 100, or 200 mg/kg/day (TWA); 5 days/week	78 weeks treatment plus 33 weeks observation	Treated animals had dose-related decrease in survival and weight gain, slight decrease in food consumption, increased severity and incidence of pulmonary lesions characteristic of pneumonia; necrosis of hepatic parenchyma NS in controls, 3/50 low dose M, 4/50 high dose M, 3/49 low dose F, 11/48 high dose F; hyperplasia of urinary bladder epithelium 1/18 control M, 7/45 low dose M, 1/45 high dose M, NS for control F, 6/43 low dose F, 2/40 high dose F; increased splenic hematopoiesis in 1/18 control M, 3/45 low dose M, 6/45 high dose M; both control and treated animals had chronic nephritis; low but statistically significant increased incidence of renal epithelial tumors in treated M (see Carcinogenicity section)	NCI, 1976

TABLE 5-4

Effects of Subchronic or Chronic Oral Administration of Chloroform to Animals (cont.)

Species, Strain Age/Weight at Start	Sex	No. at Start	Vehicle	Dosage	Duration	Response	Reference
rats, Sprague-Dawley NS	M,F	10/sex/dose level	toothpaste, gavage	0, 15, 30, 150, or 410 mg/kg/day 6 days/week	13 weeks	At 410 mg/kg/day, increased liver weight with fatty change and necrosis, gonadal atrophy, increased cellular proliferation in bone marrow; at 150 mg/kg/day, changes less pronounced but effect (NS) on relative liver and kidney weights; presumably no effects at lower dosage levels	Palmer et al., 1979
rats, Sprague-Dawley SPF. 180 to 240 g M 130 to 175 g F	M,F	50/sex/group	toothpaste, gavage	0 or 60 mg/kg/day 6 days/week	80 weeks exposure plus 15 weeks observation	Survival of treated animals slightly better than that of controls (32% treated M, 22% control M, 26% treated F, 14% control females survived to 95 weeks); body weights of treated rats slightly and progressively depressed; intercurrent respiratory and renal disease in all groups; minor histological changes in livers but no evidence of "treatment-related toxic effect" in livers; decrease ($p<0.01$) in relative liver weight in treated females; no gross or histologic treatment-related changes in brain; possible effect (NS) on incidence of severe glomerulonephritis; decrease in plasma cholinesterase in treated females	Palmer et al., 1979

TABLE 5-4 (cont.)

Effects of Subchronic or Chronic Oral Administration of Chloroform to Animals (cont.)

Species, Strain Age/Weight at Start	Sex	No. at Start	Vehicle	Dosage	Duration	Response	Reference
mice, B6C3F1, 6 weeks/19 g	F	30/group except 40 ad lib. controls	drinking water	0, 200, 400, 600, 900, 1800, or 2700 ppm in drinking water ad lib. corresponding to intakes[b] of ≈0, 32, 64, 97, 145, or 290 mg/kg/day; additional 0 ppm group matched with 2700 ppm group for water consumption	10 mice/group killed at 30, 60, and 90 days of exposure	Dose-related signs of depression during first week only; marked reduction in water consumption in higher-dose groups during first 2 weeks; body weight losses ($p<0.05$) in 97, 145, and 290 mg/kg groups and in matched controls during first week; mild hepatic centrilobular fatty change in 64, 97, 145, and 290 mg/kg groups at 30 days, but only in 2 highest dosage groups at 60 and 90 days; increase in liver fat/liver weight ($p<0.05$) for 290 mg/kg group at all 3 periods; no other treatment-related changes in serum enzyme levels, urinalysis values, or gross or microscopic appearance of tissues including kidney	Jorgenson and Rushbrook, 1980
mice, B6C3F1 35 days/18g M, 17 g F	M,F	50/sex/dose level; 20/sex matched controls	corn oil, gavage	M: 0, 138, or 227 mg/kg/day; F: 0, 238, or 447 mg/kg/day; 5 days/week	78 weeks treatment plus 14 to 15 weeks observation	Survival decreased in high dose F, unaffected in other treated groups; high incidences of hepatocellular carcinoma in treated mice (see Carcinogenicity section); renal inflammation in 10/18 control M, 2/50 low dose M, 1/50 high dose M	NCI, 1976

TABLE 5-4

Effects of Subchronic or Chronic Oral Administration of Chloroform to Animals (cont.)

Species, Strain Age/Weight at Start	Sex	No. at Start	Vehicle	Dosage	Duration	Response	Reference
mice, Schofield	M,F	10/sex per dosage	toothpaste, gavage	0, 60, 150, or 425 mg/kg/day; 6 days/week	6 weeks	At 425 mg/kg, 100% mortality; at 150 mg/kg, 8/10 M died and weight gain of F markedly retarded; at 60 mg/kg, weight gain of both sexes moderately retarded; no other observations mentioned	Roe et al., 1979
mice, ICI (expt. 1) ICI (SPF) (expt. 2) ICI, CBA, C57BL, CF/1 (expt. 3) ≤10 weeks old	expt. 1: M,F expt. 2 and 3: M treated and control, plus some F control	treated: 52/sex/dose level; control: 52 to 206/sex/strain/vehicle plus untreated	toothpaste in all 3 expt. for all strains and sexes plus arachis oil in expt. 3 for ICI, gavage	0, 17 (expt.1), or 60 mg/kg/day, 6 days/week	80 weeks treatment; 16 to 24 weeks observation according to numbers of survivors	Survival generally better in 60 mg/kg groups than in controls except for CF/1 animals or when chloroform given in arachis oil; slight retardation of weight gain in 60 mg/kg groups; no effect on hematologic values (tested in expt. 2 only); no treatment-related adverse effect on liver or other tissues except in kidneys as follows: 60 mg/kg in toothpaste - increased incidence of moderate to severe renal changes ($p < 0.001$) in CBA and CF/1 M, 60 mg/kg in oil-increased incidence of moderate to severe kidney disease ($p < 0.05$) in 1C1 M; increased incidence of benign and malignant kidney tumors in ICI M treated with 60 mg/kg in toothpaste or oil (see Carcinogenicity section)	Roe et al., 1979

TABLE 5-4

Effects of Subchronic or Chronic Oral Administration of Chloroform to Animals (cont.)

Species, Strain Age/Weight at Start	Sex	No. at Start	Vehicle	Dosage	Duration	Response	Reference
dogs, beagle 18 to 24 weeks 7 to 8 g	M,F	1/sex/dose level for 90 and 120 mg/kg; 2/sex/ dose level for lower dosages	toothpaste in gelatin capsule, orally	30, 45, 60, 90, or 120 mg/kg/day, 7 days/week	13 weeks for 30 and 45 mg/kg, 18 weeks for 60 mg/kg, 12 weeks for 90 and 120 mg/kg	No deaths; occasional vomiting; marked weight loss in all dogs and poor general condition in some at 60 mg/kg or higher; apparent suppression of appetite initially at all dosages and throughout at 60 mg/kg and higher; jaundice and increased SAP, SGOT, SGPT, bilirubin, and ICD values in male at 120 mg/kg; increased SGPT values in 4/4 and increased SAP and SGOT values in 2/4 at 60 mg/kg; hepatocyte enlargement and vacuolation with fat deposition at 60 mg/kg and higher; discoloration of liver, increased liver weight, and slight fat deposition in hepatocytes at 45 mg/kg; no effect on any of these clinical chemistry or histological parameters at 30 mg/kg	Heywood et al., 1979

TABLE 5-4

Effects of Subchronic or Chronic Oral Administration of Chloroform to Animals (cont.)

Species, Strain Age/Weight at Start	Sex	No. at Start	Vehicle	Dosage	Duration	Response	Reference
dogs, beagle, 8 to 24 weeks, 7 to 8 kg	M,F	8/sex/dose level; 16/sex vehicle controls; plus other controls	toothpaste in gelatin capsule, orally	0, 15, or 30 mg/kg/day; 6 days/week	7.5 years treatment plus 20 to 24 weeks observation	No effect on survival, growth, organ weights, hematologic or urinalysis values (checked at intervals throughout); moderate dose-related elevation of SGPT reaching peak in sixth year of study, reverting to normal levels after treatment discontinued; other serum enzyme indicators of hepatic damage (checked during the latter portion of the study) followed pattern similar to SGPT, but BSP retention and ICD values were unaffected; aggregation of vacuolated histiocytes ("fatty cysts") in livers of all groups but cysts were larger and more numerous in treated dogs and persisted after treatment ended; fat deposition affected more renal glomeruli in 30 mg/kg group than in other groups	Heywood et al., 1979

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[a] Calculated by Jorgenson and Rushbrook (1980) from measured average body weights and water consumption.

[b] Calculated from Jorgenson and Rushbrook's statement that the mice had actual intake levels of from 148 to 175% of the intended levels of 20, 40, 60, 90, 180, and 270 mg/kg/day.

[c] Calculated by Chu et al. (1982b) by multiplying the fluid intake volume by the concentration of chloroform

[d] Growth rate data were given only for the highest dose and mg/kg/day were calculated from this information by taking average weights over the 90-day period of exposure.

SPF = specific pathogen-free; SGPT = serum glutamic-pyruvic transaminase; SAP = serum alkaline phosphatase; BSP = bromsulphthalein; ICD = isocitric dehydrogenase (serum)

Purity of the chloroform samples used in all these studies was generally high and is discussed in the section on Carcinogenicity.

M = male; F = female; TWA = time-weighted average dose for days on which chemical was administered; NS = Not specified;

and mild hepatic changes in mice and rats (Jorgenson and Rushbrook, 1980; Palmer et al., 1979), hepatic damage in dogs (Heywood et al., 1979), and renal damage in male mice of some sensitive strains (Roe et al., 1979), and in dogs (Heywood et al., 1979). In addition to hepatic damage, ingestion of ≈300 mg/kg/day of chloroform produced thyroid lesions in rats (Chu et al., 1982b). In one study, a decreased incidence of renal inflammation occurred in male mice treated with chloroform at 138 or 227 mg/kg/day, 5 days/week (NCI, 1976). A similar effect may have occurred in rats (Palmer et al., 1979), but the authors did not specify whether the effect of chloroform was to increase or decrease the incidence of intercurrent renal disease. The NCI (1976) report stated that hepatic necrosis, hyperplasia of the urinary bladder epithelium, and increased splenic hematopoiesis in rats may have been related to chloroform treatment, but incidences in controls for some of these effects were not reported and the data, shown in Table 5-4, are difficult to interpret. Many of the studies summarized in Table 5-4 were at least partially designed as investigations of carcinogenicity and, hence, are also discussed in the carcinogenicity section of this document; some experimental details are discussed more fully in that section.

From the data presented in Table 5-4, it appears that rats and mice can tolerate higher daily intakes of chloroform when it is given in their drinking water or in a toothpaste base (by gavage) than they can when the chemical is administered in corn or arachis oil (by gavage). In the subchronic study of Jorgenson and Rushbrook (1980), rats and mice appeared to adapt to low levels of chloroform intake (up to ≈100 mg/kg/day); signs of depression and mild hepatic damage that occurred initially had disappeared by 90 days of treatment. Elevated indices of liver damage (e.g., SGPT levels) in dogs chronically exposed to chloroform reverted to "normal" after treatment was discontinued, although histological changes persisted. Similarly, the mild liver and thyroid lesions seen in rats exposed to high doses of chloroform via their drinking water for 90 days were no longer apparent in rats allowed to recover for an additional 90 days (Chu et al., 1982b).

5.3 INVESTIGATION OF TARGET ORGAN TOXICITY IN EXPERIMENTAL ANIMALS

5.3.1 Hepatotoxicity. An extensive review of the early literature dealing with chloroform-induced liver damage by von Oettigen (1964) notes studies beginning in 1891. More recently, Groger and Grey (1979) summarized reports describing chloroform-induced liver hepatotoxicity as follows: typical effects of chloroform on liver cells are extensive vacuolization, disappearance of glycogen, fatty degeneration, swelling, and necrosis, all starting in the centrilobular areas. There is also often hemorrhaging into the parenchyma and infiltration of polymorphonuclear cells and monocytes. Electron-microscopic observations of liver parenchymal cells from chloroform-intoxicated rats as carried out by Scholler (1966, 1967) revealed deposition of lipid droplets in the cytoplasm, partial destruction of the mitochondrial matrix, proliferation of smooth endoplasmic reticulum, and swelling of the rough endoplasmic reticulum with detachment of ribosomes.

Kylin et al. (1963) conducted a study of the hepatotoxic effects of inhaled trichloroethylene, tetrachloroethylene, and chloroform in mice with the objective of finding the lowest concentration of the substances producing signs of liver damage after a single 4-hour exposure period. Histological examination showed that a concentration of 100 ppm caused moderate fatty infiltration in mice killed 1 day after exposure. At $\geq$200 ppm, the extent of the alteration increased with concentration and was more pronounced after 1 day than 3. Thus, judging from the histological picture, the smallest concentrations (ppm) of the different agents to produce more severe alterations in the exposed group than in the controls were as follows:

	Trichloroethylene (ppm)	Tetrachloroethylene (ppm)	Chloroform (ppm)
1 day after exposure	1600-3200	<200	<100
3 days after exposure	>3200	200 to 400	100 to 200

On this basis, the hepatotoxic effects of trichloroethylene, tetrachloroethylene, and chloroform are in the approximate ratios 1:10:20. The amount of liver fat was raised at 400 ppm of chloroform. A third indicator of liver toxicity was an increase in serum ornithine carbamyl transferase (S-OCT) activity at 24 hours in animals exposed to 200, 400, and 800 ppm of chloroform.

A study of the effect of oral doses of chloroform on the extent of liver damage in white mice ("of a Swiss strain") was conducted by Jones et al. (1958). Minimal changes characterized by midzonal fatty infiltration were observed 72 hours after the administration of 30 mg (0.02 mℓ)/kg. When the dose was increased to 133 mg (0.09 mℓ)/kg, a massive fatty infiltration of the total liver lobule was found. At a level of 355 mg (0.24 mℓ)/kg, massive fatty infiltraton occurred along with severe central lobular necrosis. Information on the hepatotoxicity of long-term chloroform administration has been presented in the sections on Effects of Chronic Exposure to Chloroform. Inhalation exposure of rats to 25, 50, or 85 ppm chloroform for 7 hours/day, 5 days/week for 6 months produced centrilobular granular degeneration and focal necrosis in their livers. In subchronic studies, ingestion of up to ≈100 mg/kg/day of chloroform produced mild, transient histological changes in the livers of rats and mice (Jorgenson and Rushbrook, 1980), ingestion of 145 or 190 mg/kg/day produced fatty change in the livers of mice (Jorgenson and Rushbrook, 1980), and administration of 410 mg/kg/day by gavage produced fatty change and necrosis in the livers of rats. Dogs treated subchronically with 45 mg/kg/day by the oral route had histological evidence of slight hepatic fatty change, with increasingly severe changes noted at dosages of 60 and 120 mg/kg/day.

In chronic oral studies, rats had minor histological change in their livers and a decrease in relative liver weights when given 60 mg/kg/day of chloroform, 6 days/week, while the livers of mice were unaffected at this dosage. Dogs had some evidence of liver damage (clinical chemistry parameters) and an increase in the number and size of fatty cysts in their lifetime when administered 15 or 30 mg/kg/day orally for 6 days/week. The mechanism by which chloroform exerts its hepatotoxic effects has been widely investigated and efforts have been made to identify the responsible metabolite(s).

As long ago as 1928, it was suspected that the liver damage induced by chloroform may be due not only to the chemical itself, but might be caused by a degradation product (Lucas, 1928). The concept that chloroform is excreted unchanged was disproved since a large number of studies (Butler, 1961; Paul and Rubenstein, 1963; Van Dyke et al., 1964; and Reid and Krishna, 1973) indicated that the tissue necrosis induced by chloroform is associated with the covalent binding of toxic metabolites and alkylation of tissue proteins. Autoradiograms have revealed that this binding occurs predominantly in the necrotic areas (Illet et al., 1973). It was also shown by McLean (1970) that pretreatment of rats with phenobarbital (a microsomal enzyme inducing agent) greatly enhances the lethality of chloroform.

Brown et al. (1974) proposed a mechanism of chloroform hepatotoxicity implicating a free radical metabolite which can react with glutathione (GSH) (a tripeptide which protects against hepatotoxicity), diminishing GSH levels in the liver. According to this hypothesis, once GSH levels are depleted, further metabolism would result in the reaction of the metabolite with microsomal protein, and hence, necrosis. This proposal was based on observations in phenobarbital pre-treated rats anesthetized with chloroform, that hepatotoxicity was enhanced and GSH levels were decreased by the induction of microsomal enzymes. Covalent binding of chloroform metabolites to microsomal proteins in vitro was also enhanced by enzyme induction, an effect prevented by GSH. Similar findings were reported in mice by Ilett et al. (1973), who found severe chloroform-induced centrilobular necrosis in phenobarbital pretreated mice, but only slight centrilobular damage in mice exposed only to chloroform.

Thus, the hepatotoxicity of chloroform appears to depend on 1) the rate of its biotransformation to produce reactive metabolite(s), and 2) by the amount of GSH available to conjugate with and thus inactivate the metabolite(s).

The role of GSH in chloroform-induced hepatotoxicity was further studied by Docks and Krishna (1976), who found that only thoses doses of chloroform that decreased liver GSH caused liver necrosis when administered to phenobarbital pretreated rats.

More recently, Ekström et al. (1982) studied the mechanism of GSH depletion by chloroform in rats pretreated with phenobarbital. The synthesis of GSH proceeds via two enzymatic steps, the first of which is rate limiting:

$$\text{glutamate} + \text{cysteine} \xrightarrow[\text{synthetase}]{\gamma\text{-glutamyl-cysteine}} \text{dipeptide}$$

In the presence of glycine, the reaction continues via GSH synthetase to produce GSH. When the soluble fraction from livers of rats sacrificed at various times after chloroform exposure was incubated in the presence of these amino acids, it was found that GSH synthesis was inhibited within 4-6 hours, while liver necrosis was evident only after 6 hours. When glycine was eliminated from the initial part of the incubation, the dipeptide accumulated, but at a lower rate in the presence of chloroform than in its absence. Later addition of glycine resulted in GSH synthesis at a rate similar to control values. Thus, it appears that chloroform, or rather a reactive metabolite, inhibited GSH synthesis at the rate limiting step (i.e., the formation of dipeptide by γ-glutamyl-cysteine synthetase).

The biotransformation of chloroform (as discussed in Chapter 4) depends on the activity of the microsomal drug metabolizing enzymes. Substances that induce these enzymes were shown to, indeed, enhance the hepatotoxicity of chloroform as evidenced by increased serum glutamic-pyruvic transaminase (SGPT) levels and decreased hepatic glucose-6-phosphatase activity (Lavigne and Marchand, 1974). An inhibitor of the drug metabolizing enzymes SKF-525A, however, while increasing the excretion of ^{14}C-carbon monoxide in rats administered ^{14}C-labelled chloroform, failed to diminish the hepatotoxicity of chloroform, leading these authors to conclude that factors other than metabolism may be involved.

McMartin et al. (1981) demonstrated that altering the cytochrome P-450 concentrations in the livers of chloroform-exposed rats also altered the hepatotoxicity, as measured by the incidence of hepatic lesions and by serum alanine aminotransferase activities. Both fasting and phenobarbital pretreatment increased the cytochrome P-450 content and liver damage, while cadmium produced the opposite effect.

Theories of chloroform hepatotoxicity involve the formation of reactive intermediates by liver enzymes. How these intermediates exert their hepatotoxic effect has been the subject of several studies. It has been suggested by Masuda et al. (1980) that, based on the chloroform-induced indices of hepatotoxicity of decreased microsomal glucose-6-phosphatase activity and cytochrome P-450 content with increased hepatic malondialdehyde levels, the lipid peroxidation hypothesis proposed for carbon tetrachloride may also apply to the case of chloroform. Qualitative and mechanistic differences of hepatotoxicity between the two chemicals were noted, however.

The interactive hepatotoxicity of chloroform and carbon tetrachloride was studied by Harris et al. (1982) who found that, while neither chemical alone given at subthreshold dose altered SGPT activity, hepatic triglyceride content, or hepatic calcium content, when given together, these chemicals increased the toxic response in rats. Administration of either or both chemicals had no effect on GSH levels or conjugated diene formation, but ethane expiration was increased in rats given both chemicals. Diene conjugation and ethane expiration are indices of lipid peroxidation. Histopathological changes were more severe from the combination than from either chemical alone. Although the mechanism of the hepatotoxic interaction between chloroform and carbon tetrachloride is unclear, the authors suggested that there might be a combined effect of phosgene formation and lipid peroxidation initiation.

It should be noted that the prevailing theories implicate phosgene as the major metabolite responsible for chloroform hepatotoxicity (Reynolds and Yee, 1967; Sipes et al., 1977; Mansuy et al., 1977; Pohl et al., 1977). Other potential toxic metabolites discussed by Pohl (1979) in a review of this subject are a trichloromethyl radical and dichlorocarbene; however, they are considered less important than phosgene in this regard.

A study by Stevens and Anders (1981) supports the phosgene-mediated mechanism. The time course of changes in SGPT levels and covalent binding of ^{14}C to proteins was examined in microsomal and soluble fractions from phenobarbital-pretreated rats sacrificed at various times after chloroform or ^{14}C-chloroform administration. It was found that ^{14}C binding was maximal at 6 hours while indices of liver damage peaked at 18 hours after chloroform exposure. Further experiments were performed in which diethyl maleate (a GSH depletor) treatment caused increased ^{14}C-binding to soluble and microsomal fractions and increased SGPT levels, perhaps by inhibiting the metabolism of phosgene to carbon monoxide or stable conjugates. Cysteine, which reacts with phosgene to produce 2-oxothiazolidine-4-carboxylic acid, had a protective effect. Diethyl maleate also diminished, but did not eliminate the deuterium isotope effect on the GSH dependent chloroform metabolism to carbon monoxide, which would be expected if carbon monoxide formation occurred subsequent to phosgene production. Thus, the hepatotoxicity of chloroform can be altered by altering the various reaction pathways of phosgene, strongly indicating that phosgene is the toxic intermediate.

From the above discussion, it appears that, to be hepatotoxic, chloroform must first be metabolized by microsomal drug metabolizing enzymes to an active intermediate, probably phosgene, which in turn can react by various pathways, depending on GSH levels, one of which is the covalent binding to liver proteins resulting in necrotic lesions.

5.3.2 Nephrotoxicity

As noted by Watrous and Plaa (1972), the extensive body of research on the hepatotoxicity of halogenated hydrocarbons has tended to overshadow the fact that some of these agents are also nephrotoxic. Earlier reports of chloroform nephrotoxicity include those of Heller and Smirk (1932), who found that rats anesthetized with chloroform showed a diminished ability to excrete a water load given prior to anesthesia, and Knocher and Mandelstam (1944), who noted that chloroform injection produced a fatty infiltration of the kidney.

Renal necrosis produced by the oral administration of chloroform was described by Eschenbrenner and Miller (1945a). The necrosis, observed only in male mice, involved portions of both proximal and distal convoluted tubules. The nuclei of the epithelial cells were often absent or fragmented and the cytoplasm was coarsely granular and deeply eosinophilic. The glomeruli and collecting tubules appeared normal.

Sex and strain differences in the sensitivity of mice to chloroform nephrotoxicity were further studied by Deringer et al. (1953). Exposure of strain C3H mice to air containing ≈5 mg/ℓ of chloroform for 1, 2, or 3 hours resulted in lesions of the kidneys of all of the males but in none of the females. In animals dying within 1 day after exposure, epithelium of the proximal tubules and portions of the distal tubules were generally necrotic. The lumens of those segments of tubules were dilated. The glomeruli were relatively unaffected. The mice dying or sacrificed at later time intervals exhibited calcification in the necrotic area.

Similar lesions were found in males of strains C3H, C3Hf, A, and HR. However, strains C57BL, C57BR/cd, C57L, and ST were resistant to chloroform-induced nephrotoxicity.

Comparable results were reported by Krus and Zaleska-Rutczynski (1970). Subcutaneous administration of chloroform to C3H/He male mice resulted in renal tubular necrosis, with death ensuing 4-9 days later. The lesions were calcified with no evidence of regeneration. Female mice of this strain, males and females of the C57BL/6JN and BN strains, and F_1 generation males of the cross of female C3H/He with male C57BL/6JN mice survived the administration of chloroform (0.1 mℓ of 0.05 g chloroform in 1 mℓ ethyl laureate). Additional studies were performed with males and females of this F_1 generation and the resistant BN strain, in which animals were sacrificed at various time after chloroform administration. All mice survived, while all female mice were resistant, showing no kidney lesions at any time in the experiment. Renal damage was morphologically apparent in all male mice by 12 hours, but regeneration developed by day 4 and continued until the end of the experiment. It was concluded that although all male mice had tubular lesions, the ones surviving had tubules that did not calcify and a large degree of renal regeneration.

Several investigators have studied the influence of testosterone on chloroform-induced renal damage. Eschenbrenner and Miller (1945b) performed experiments in which they saw extensive necrosis of portions of the proximal and distal renal tubules in normal male and in testosterone-treated castrated male mice following the acute oral administration of chloroform. However, no necrosis was found after chloroform was administered to female mice or castrated male mice. Continuing this line of investigation, Culliford and Hewitt (1957) reported the following results:

1) Adult male mice of two strains (CBA and WH) developed extensive necrosis of the renal tubules after exposure to low concentrations of chloroform vapor (7-10 mg/ℓ for 2 hours). Adult females showed no renal damage after equivalent exposure.

2) Adult females became fully susceptible to necrosis after treatment with androgens. The susceptibility of males was greatly reduced by treatment with estrogens.

3) Castration removed the susceptibility of the males of one strain, but did not completely remove it in another. The residual susceptibility of castrates was abolished by adrenalectomy.

4) Male mice under 11 days old were not susceptible to necrosis even after massive doses of androgen. Between 11 and 30 days, they were susceptible if given androgen. Thereafter, they became spontaneously susceptible.

5) Liver damage occurred in nearly all exposed mice and was not correlated with sex hormone status.

6) Susceptibility could be induced in gonadectomized mice by methyl testosterone, testosterone propionate, dehydroepiandrosterone, progesterone, and large doses of cortisone acetate.

Hill (1978) also performed experiments demonstrating similar strain and sex differences in chloroform-induced renal toxicity. The renal toxicity of a fixed oral dose of chloroform to castrated male mice was increased with increasing doses of administered testosterone. Plasma levels of testosterone in resistant strains tended to be lower than levels in susceptible strains. Hill (1978) conjectured that a testosterone may act by sensitizing the renal proximal convoluted tubules to chloroform through a testosterone receptor mechanism.

Eschenbrenner and Miller (1945b), however, linked susceptibility to the nephrotoxic action of chloroform to differences in kidney morphology and physiology induced by testosterone.

Information on the nephrotoxicity of long-term chloroform administration has been presented in the section on Effects of Chronic Exposure to Chloroform. Inhalation of 25, 50, or 85 ppm of chloroform 7 hours/day, 5 days/week for 6 months produced cloudy swelling of the renal tubular epithelium in rats. Male mice of certain sensitive strains had increased incidences of moderate to severe renal disease when treated orally with chloroform at a dosage of 60 mg/kg/day, 6 days/week in a chronic study (Roe et al., 1979). Chronic oral administration of 30 mg/kg/day of chloroform, 6 days/week, to dogs produced an increase in the numbers of renal glomeruli affected by fat deposition (Heywood et al., 1979).

The mechanism of the nephrotoxicity of chloroform has been less extensively studied than has that of hepatotoxicity. Ilett et al. (1973) suggested that the hepatotoxic metabolite produced in the liver is transported via the circulation to the kidney where it exerts its nephrotoxic effects. More recent studies (McMartin et al., 1981; Kluwe and Hook, 1981) suggest that chloroform may also be metabolized in the kidney, but by a different mechanism.

McMartin et al. (1981) altered the concentrations of cytochrome P-450 by fasting, by phenobarbital pretreatment, or by administration of cadmium to rats given chloroform as a challenge. Fasting increased cytochrome P-450 concentrations in both liver and kidney, and chloroform-induced damage was enhanced in both organs of fasted animals. Pretreatment with cadmium decreased cytochrome P-450 in livers but not kidneys and significantly diminished liver damage due to chloroform while having no effect on kidney damage due to chloroform exposure. Phenobarbital pretreatment resulted in increased liver but not kidney cytochrome P-450 and, likewise, chloroform-induced damage was enhanced in livers but not kidneys. Thus, pretreatments that altered hepatic cytochrome P-450 levels had no effect on chloroform-produced renal effects, suggesting that a metabolite of chloroform, which is responsible for kidney damage, is produced in the kidney.

The mechanism of chloroform nephrotoxicity was also investigated by Kluwe and Hook (1981), who found no difference between nephrotoxicity and hepatotoxicity with respect to the effects of microsomal enzyme inhibitors and diethyl maleate. Mice were injected intraperitoneally with chloroform either before piperonyl butoxide or SKF-525-A administration or after diethyl maleate, piperonyl butoxide or SKF-525A exposure. Although it inhibits microsomal enzymes, SKF-525-A, administered either before or after chloroform administration, did not reduce the hepatotoxicity or the nephrotoxicity of chloroform. This is consistent with a similar finding by Lavigne and Marchand (1974). Piperonyl butoxide, when given before chloroform, protected against toxicity in both organs, but when given after chloroform, did not. This finding indicated that an enzymatic step in the metabolism of chloroform by both organs was inhibited. The effect of diethyl maleate was to enhance the toxicity of chloroform in both organs. Thus, the mechanism of chloroform nephrotoxicity appears to be similar to that of hepatotoxicity with respect to these substances.

5.4 FACTORS MODIFYING THE TOXICITY OF CHLOROFORM

From the preceding discussion, it is evident that the alterations of microsomal enzyme activity or hepatic GSH levels influence the severity of toxic effects induced by a given amount of chloroform. It follows then that many factors could alter chloroform toxicity by affecting these parameters or acting through other mechanisms. These substances are of interest because they fall into categories of accidental or intentional exposure to humans. Alcohol, dietary components, pesticide, and steroids are some of the substances which are discussed below.

5.4.1 Factors that Increase the Toxicity. The effect of ethanol pretreatment on chloroform-induced hepatotoxicity in mice was studied by Kutob and Plaa (1962a). An intoxicating dose (5 g/kg) of ethanol was administered orally to mice daily for 15 days initially, with a systematic shortening of the duration to a single exposure. A challenging dose of chloroform (0.08 mℓ/kg) was administered subcutaneously either 12, 15, or 24 hours after ethanol treatment. Liver dysfunction was measured by prolongation of phenobarbital sleeping time, bromsulphalein (BSP) retention, liver succinic dehydrogenase activity, and histological examination. Regardless of the ethanol treatment period, phenobarbital sleeping time was significantly increased in mice receiving ethanol followed by chloroform when compared with mice receiving either substance alone. Similar findings were found for BSP retention. The in vitro succinic dehydrogenase activity was significantly reduced by ethanol pretreatment followed by chloroform administration 12 or 24 hours, but not 48 hours, later, when compared with activities from mice receiving only chloroform. Histological changes were seen in the livers of mice given ethanol 15 hours to 4 days prior to chloroform challenge, while mice receiving either chemical alone had morphologically normal livers. It was also determined that the ethanol treatment increased liver triglyceride content, with a maximum at 15 hours, and that ethanol pretreatment significantly increased the concentration of chloroform in the livers with a maximum at 12 hours after chloroform challenge. From these results, it was noted that a single dose of ethanol was just as effective as multiple doses. A mechanism was proposed for the ethanol enhanced chloroform-induced hepatotoxicity in which ethanol increases liver lipid content (as evidenced by increased triglycerides) resulting in increased concentrations of chloroform to be metabolized in the liver.

In support of this mechanism is the observation that oral isopropanol pretreatment for 5 days (0.3 mℓ/100 g for 2 days and 0.15 mℓ/100 g for 3 days) followed 12 hours later by 5 daily inhalations of chloroform (5000 ppm first day, 2500 ppm on the next four days), 2 hours/day led to severe fatty infiltration of the liver. Chloroform alone increased the pool of triglycerides (Danni et al., 1981).

In contrast, Sato et al. (1980) studied the mechanism by which ethanol enhances hydrocarbon metabolism, including that of chloroform. Rats ingesting ethanol in their drinking water for 3 weeks were sacrificed 10 hours after the final exposure. Control rats were given isocaloric glucose solutions. Liver microsomal enzyme systems were prepared and liver protein and cytochrome P-450 contents analyzed, the increased contents being indicative of microsomal enzyme synthesis in response to alcohol. When chloroform was added as a substrate, its metabolism was enhanced by 6 times, much more than could be accounted for by enzyme induction alone. Microsomes prepared from rats that were withdrawn from ethanol 24 hours prior to sacrifice did not show enhanced activity. In a subsequent study (Sato et al., 1981), rats receiving a single gavage dose of 0, 2, 3, 4, or 5 g/kg ethanol were sacrificed 18 hours later. The _in vitro_ metabolism of chloroform by microsomes prepared from these rats was enhanced very little at 2 g/kg, slightly more at 3 g/kg, and dramatically at 4 g/kg. At 5 g/kg, however, enhanced enzyme activity was no greater than at 3 g/kg ethanol. When ethanol was added directly to the incubation system, the metabolism of chloroform was inhibited. Rats receiving 5 g/kg ethanol retained relatively large amounts in the blood and liver, while those receiving 4 g/kg retained almost none. If the ethanol remaining in the rats exerted an inhibitory effect on enzyme activity, then microsomal enzymes prepared from 5 g/kg ethanol-treated rats, mixed with the soluble fraction from control rats, should show increased activity when compared with both microsomal and soluble fractions from ethanol-treated rats. This was found to be the case.

Based on these results and studies on metabolism of other hydrocarbons in vitro and in vivo, Sato et al. (1981) suggested that ethanol is both a stimulator and an inhibitor of drug metabolizing enzymes, depending on how much ethanol remains in the body and thus how much time has elapsed since ethanol ingestion. Thus, when ethanol is first ingested, it acts as a competitive inhibitor of microsomal enzyme activity, but as it disappears from the body, an optimum for stimulation may be reached and metabolism enhanced. It was postulated that since the metabolism of chloroform was enhanced to a much greater extent than can be explained from enzyme induction alone, perhaps ethanol modifies the enzyme activities by other mechanisms such as modification of membrane properties, allosteric effects, or by displacement of substrate already bound.

Polybrominated biphenyls (PBBs) have also been found to potentiate the toxicity of chloroform (Kluwe and Hook, 1978). Mice were fed diets containing 0, 1, 25, or 100 ppm PBB for 14 days. One day before sacrifice, the mice were given a single intraperitoneal injection of 0, 0.5, 2.5, 5.0, or 50 $\mu\ell$/kg chloroform. PBB enhanced the toxicity of chloroform in both the liver and the kidney as evidenced by results of blood urea nitrogen (BUN) and serum glutamic oxaloacetic transaminase (SGOT) determinations and by inhibition of p-aminohippuric acid (PAH) uptake by renal slices. PBB also reduced the LD_{50} of chloroform in these mice and the deaths were attributed to hepatic necrosis. Since PBBs were known to induce the drug metabolizing enzymes, their effects on chloroform were assumed to be due to enhanced chloroform metabolism.

Steroids appear to play a role in the potentiation of chloroform toxicity, especially in the kidney as seen from the sex-related differences in the response of mice (Eschenbrenner and Miller, 1945a; Deringer et al., 1953) and by experiments involving testosterone administration to castrated male mice (Eschenbrenner and Miller, 1945b; Culliford and Hewitt, 1957; Hill, 1978) discussed previously. Clemens et al. (1979) further studied this phenomenon in castrated male and intact female mice. Dose-dependent testosterone sensitization of renal tubules to a fixed dose of chloroform was observed in castrated males with the response ranging from kidney dysfunction to death at high doses. The androgenic progestin, medroxyprogesterone acetate, enhanced chloroform-induced kidney damage in both castrated males and intact females. Progesterone or hydrocortisone potentiated chloroform toxicity in DBA/2J castrated male mice, but not in the C57BL/J6 strain males nor in any of the females. The mechanism by which the androgens exerted their potentiation may have been mediated through strain specific androgen receptors of the proximal convoluted tubular cells. The mechanism for the potentiating action of the other steriods was less clear.

The potentiation of chloroform toxicity by ketones and ketogenic substances has been studied extensively in recent years. Hewitt et al. (1979) and Cianflone et al. (1980) found that while pretreatment of mice with the insecticide, kepone (a ketone), enhanced the liver damage caused by chloroform exposure, the structurally related mirex (a non-ketone) did not. Other ketones were compared for their ability to enhance the hepatotoxic and nephrotoxic action of chloroform in rats with the following results: methyl n-butyl ketone and 2,5-hexanedione were the most potent enhancers, followed by acetone, followed by n-hexane (a ketogenic chemical) (Hewitt et al., 1980). Jernigan and Harbison (1982) studied the potentiation by 2,5-hexanedione of chloroform hepatotoxicity in mice with specific reference to sex differences. Female mice were more susceptible to the dose-dependent enhancement of chloroform hepatotoxicity as well as to the dose-related increase in hepatic microsomal enzymes. Pretreatment of male mice with 2,5-hexanedione potentiated the toxicity of deuterated chloroform but to a lesser extent than chloroform; however, this deuterium isotope effect did not occur in female mice in vivo. Phenobarbital pretreatment did elicit a deuterium isotope effect in female mice in vivo, suggesting that 2,5-hexanedione pretreatment altered chloroform metabolism by a different mechanism than did phenobarbital. Jernigan and Harbison (1982) speculated that perhaps female mice have greater microsomal enzyme activities, different membrane properties, or perhaps produce a different reactive metabolite of chloroform than do males.

The mechanism of ketone potentiation of chloroform-induced hepato- and nephrotoxicity was also investigated by Branchflower and Pohl (1981) using methyl *n*-butyl ketone (MBK). Male rats were pretreated with MBK followed by chloroform administration. The metabolism of chloroform by liver and kidney microsomal enzymes and the toxicity to these organs were examined. Control experiments were conducted in which rats were either not pretreated, not given chloroform, or given deuterated chloroform ($CDCl_3$) instead of chloroform. MBK increased cytochrome P-450 levels and NADPH-dependent cytochrome reductase activity in liver microsomes, while having no effect on renal levels of these microsomal components. MBK pretreatment doubled the rate of metabolism of chloroform to diglutathionyl dithiocarbonate (GSCOSG) in microsomal preparations, and more GSCOSG was excreted into the bile of the pretreated animals when compared with rats receiving only chloroform. The amount of GSCOSG in bile was less in MBK-$CDCl_3$ treated animals. GSH levels were significantly decreased by MBK treatment and this decrease was enhanced following chloroform exposure, and to a lesser extent, following $CDCl_3$ exposure. Rats pretreated with MBK followed by chloroform had greatly elevated levels of SGPT associated with liver necrosis and significantly greater BUN levels associated with renal cortical tubule lesions over the control groups. A mechanism was proposed whereby MBK, by increasing cytochrome P-450 levels, enhanced the metabolism of chloroform to phosgene. Furthermore, according to the hypothesis, the phosgene was converted to GSCOSG through GSH, levels of which were diminished by MBK, because the more phosgene formed, the more GSH was depleted in the reaction. The results with $CDCl_3$ indicated that C-H bond was involved in the mechanism. Although MBK also potentiated chloroform toxicity to the kidney, a different mechanism may have been involved since renal cytochrome P-450 and renal GSH levels were not affected.

5.4.2 Factors that Decrease the Toxicity. As discussed above, the experiments of Sato et al. (1980, 1981) indicate that ethanol is both a stimulator and an inhibitor of microsomal enzymes, and hence, of chloroform metabolism and toxicity, depending upon the length of time after ingestion.

Disulfiram and its metabolites have also been studied with respect to their protective effects of chloroform-induced hepatotoxicity (Scholler, 1970; Masuda and Nakayama, 1982). Disulfiram is used in treating chronic alcoholism and is metabolized to carbon disulfide and diethyldithiocarbamate (a herbicide) (IARC, 1976). Disulfiram, a known inhibitor of the microsomal drug metabolizing enzymes, given to rats prior to chloroform anesthesia completely prevented the elevated SGPT activity and liver necrosis observed in rats administered chloroform alone (Scholler et al., 1970). More recently, Masuda and Nakayama (1982) studied the effects of diethyldithiocarbamate and carbon disulfide pretreatment in mice challenged with chloroform. Both substances had a protective effect as measured by SGPT activity, liver calcium content, and centrilobular necrosis. Diethyldithiocarbamate and carbon disulfide decreased the activity of the drug metabolizing enzymes *in vivo* and *in vitro*, but only in the presence of NADPH, indicating that these substances must first be metabolized before exerting their inhibitory effect on chloroform metabolism. Gopinath and Ford (1975) also found that dithiocarbamate and carbon disulfide protected against chloroform hepatotoxicity in rats, and the effect was presumed to be due to suppression of the drug metabolizing enzymes.

Dietary components can also alter the toxicity of chloroform. It is a widely held opinion that low protein content of the diet decreases microsomal enzyme activities, while a high protein diet increases the activities (McLean and McLean, 1969; Nakajima et al., 1982). If this is the case, a low protein diet should protect against chloroform hepatotoxicity by inhibiting the enzymes responsible for chloroform metabolism. It was found, however, that protein depletion did not alter the toxicity of chloroform in rats given a single oral dose (McLean and McLean, 1969; McLean, 1970). If pretreated with phenobarbital or NDDT to induce microsomal enzymes, rats maintained on a standard diet were no more susceptible to chloroform-induced liver damage that were pretreated, protein-depleted rats (McLean, 1970).

More recently, Nakajima et al. (1982) studied the individual effects of protein, fat, and carbohydrate on the metabolism of chloroform in relation to its toxicity in male rats. Test diets were varied with respect to carbohydrate, protein, or fat while maintaining isocaloric contents. Microsomal enzymes were prepared and chloroform was added as a substrate. The following results were obtained: decreased food intake increased liver microsomal enzyme activities; decreased sucrose content in the diet increased the metabolic rate; varying the protein and fat content, while holding the sucrose content constant, had no effect on the metabolic rate; a carbohydrate-free diet, which contained high protein and high fat, accelerated the rate of chloroform metabolism almost as much as 1 day of food deprivation. The authors concluded that it is a high carbohydrate content, rather than a low protein content, which is responsible for the decreased microsomal enzyme metabolism of chloroform and, hence, its toxicity.

5.5 SUMMARY; CORRELATION OF EXPOSURE AND EFFECT

The purpose of this section is to delineate dose-response relationships for the systemic toxicity of chloroform.

5.5.1 Effect of Acute Inhalation Exposure. The adverse effects on humans of inhaling high concentrations of chloroform have been well documented in the course of its use as an anesthetic. Studies that define the threshold region of exposure for such effects in humans are, however, sparse at best. Experiments involving subchronic exposure of several species of animals give some information on toxicity thresholds for renal and hepatic effects, but little for CNS and none for cardiovascular effects.

The only experimental studies conducted with humans (Lehmann and Hasegawa, 1910; Lehmann and Schmidt-Kehl, 1936) involved relatively short exposures and subjective responses. The results of these studies indicate that the odor of chloroform can be perceived at about 200 ppm. Subjective CNS effects (dizziness, vertigo) apparently did not occur at 390 ppm during a 30-minute exposure but were perceived at about 900 ppm after 2-3 minutes of exposure. Subjects exposed to 1400 ppm for 30 minutes experienced tiredness and headache in addition to the above CNS symptoms. The threshold for "light intoxication" was about 4300 ppm (20 minutes). An exposure duration of 30 minutes or less is insufficient to achieve pulmonary steady state (or total body equilibrium, Section 4.2.3). Hence, longer exposures at these concentrations would be expected to cause more severe effects.

Chloroform concentrations used for the induction of anesthesia ranged from about 20,000-40,000 ppm (NIOSH, 1974; Adriani, 1970) and for the maintenance of anesthesia ranged from 1500 ppm (light anesthesia) to 15,000 ppm (deep anesthesia) (Goodman and Gilman, 1980). Continued exposure to 20,000 ppm could result in respiratory failure, direct depression of the myocardium, and death

(Section 5.1.1). Levels of exposure sufficient to produce anesthesia have also caused cardiac arrythmias and extrasystoles (Kurtz et al., 1936; Orth et al., 1951) and hepatic necrosis and fatty degeneration (Goodman and Gilman, 1980; Wood-Smith and Stewart, 1964).

Data from acute animal exposures tend to show similar CNS effects at roughly the same levels of exposure that produced these effects in humans (Lehmann and Flury, 1943). In addition, some data on the threshold for hepatic effects has been obtained for mice. Kylin et al. (1963), in experiments with female mice of an unspecified strain, found that single, 4-hour exposures to chloroform produced mild hepatic effects (increased incidence of moderate fatty infiltration) at 100 ppm. At 200 ppm, in addition to fatty infiltration, hepatic necrosis and increased serum ornithine carbamyl transferase activity occurred. (An elevation in serum levels of this enzyme indicates liver damage according to Divincenzo and Krasavage, 1974.) Further increases in fatty infiltration, necrosis, and serum enzyme activity were observed at 400 and 800 ppm. These effects appeared to be reversible because the extent of change was less severe 3 days after exposure than it was 1 day after exposure.

Damage to the kidneys of male mice of sensitive strains (e.g., C3H) has occurred at exposure levels as low as 5 mg/ℓ (1025 ppm) for 1 hour (Deringer et al., 1953). The damage consisted of necrosis of the epithelium of the proximal tubules.

5.5.2 Effects of Acute Oral Exposure. Dose-response data for acute oral exposure of humans to chloroform is limited to case reports. A fatal dose of as little as 1/3 ounce (10 mℓ) was reported (Schroeder, 1965).

A variety of dose-response data is available for acute oral administration of chloroform to animals. Single doses that were sufficient to adversely affect kidney function (measured as excessive loss of glucose and/or protein in the urine in male mice) ranged from 89-149 mg/kg in sensitive and relatively insensitive strains (Hill, 1978). At single oral doses of 1071 mg/kg, but not 756 or 546 mg/kg chloroform, increases in organ weights and mild to moderate lesions were observed in the livers and kidneys of Sprague-Dawley rats (Chu et al., 1982a). In male B6C3F1 mice, renal necrosis occurred after 20 mg/kg and focal tubular regeneration occurred after 60 or 240 mg/kg but not after 15 mg/kg (Reitz et al., 1980). A low observed adverse-effect level (LOAEL) for hepatic effects in mice can be identified from the study of Jones et al., 1958, in which 30 mg/kg caused midzonal fatty infiltration. Doses in the range of 133-355 mg/kg (Jones et al., 1958; Reitz et al., 1980) represent a FEL (Frank-Effect-Level) for hepatic damage (including centrilobular necrosis) in mice. According to Torkelson et al. (1976), rats given "as little as" 250 mg/kg chloroform "showed adverse effects" on liver and kidney as determined by gross pathological examination. Reported oral LD_{50} values for mice ranged from 119-1400 mg/kg, depending on sex, strain, and age (Kimura et al., 1971; Hill, 1978; Bowman et al., 1978). For rats, LD_{50} values of 908-2000 mg/kg have been reported (Chu et al., 1980; Torkelson et al., 1976). The lethal dose studies included both 24-hour and delayed deaths.

5.5.3 Effects of Dermal Exposure. Chloroform is irritating to the skin. It has been reported to cause degenerative changes in the renal tubules of rabbits exposed dermally to high doses under extreme conditions (1-3.98 g/kg body weight for 24 hours under an impermeable plastic cuff) (Torkelson et al., 1976). In humans, toxicity from dermal exposure is probably not important in comparison with other routes.

5.5.4 Effects of Chronic Inhalation Exposure. Limited information on the effects of long-term intermittent exposure of humans or animals to chloroform is available. A study involving a small number of workers (Challen et al., 1958) indicates that long-term exposure to 20-71 ppm (98-346 mg/m^3) for a 4-8 hour workday, with occasional brief excursions to ≈1163 ppm, may represent a LOAEL for symptoms of CNS toxicity. No evidence of liver damage or other organic lesion was detected by physical examination and clinical chemistry tests. A single report linking liver enlargement and viral hepatitis to occupational exposure to 10-200 ppm chloroform (Bomski et al., 1967) is flawed by the apparent lack of suitable controls. The available data do not define a NOAEL (no-observed-adverse-effect-level) or NOEL (no-observed-effect- level) for humans.

Experiments with several species of animals (Torkelson et al., 1976) give some information regarding the threshold region for hepatic and renal effects of inhalation exposure to chloroform (Table 5-3). The animals were exposed to chloroform 5 days/week for 6 months. Exposure to 25 ppm of chloroform for up to 4 hours/day had no adverse effects in male rats as judged by organ and body weights and probably the gross and microscopic appearance of at least the liver and kidneys, although the authors were not explicit about the latter. Exposure to 25 ppm for 7 hours/day, however, produced histopathologic changes in the livers and kidneys of male but not female rats. These changes were characterized as lobular granular degeneration and focal necrosis throughout the liver and cloudy swelling of the kidneys. The hepatic and renal effects appeared to be reversible because rats exposed in the same way, but given a 6-week "recovery period" after the exposure period, appeared normal by the criteria tested. Increasingly pronounced changes were observed in the livers and kidneys of both sexes of rats exposed to 50 or 85 ppm. Hematologic, clinical chemistry, and urinalysis values, tested at the two higher levels of exposure, were "within normal limits."

Similar experiments with guinea pigs and rabbits gave somewhat inconsistent results. Histopathological lesions were observed in liver and kidneys of both species at 25 ppm but not at 50 ppm in either species and not in guinea pigs even at 85 ppm (Torkelson et al., 1976).

The experiments of Torkelson et al. (1976) indicate that subchronic exposure to 25 ppm (123 mg/m^3), 4 hours/day, 5 days/week represents a NOAEL and exposure to 25 ppm, 7 hours/day, 5 days/week respresents a LOAEL for rats. Guinea pigs and rabbits may be slightly less sensitive.

5.5.5 Effects of Chronic Oral Exposure. Little dose-response data for oral exposure of humans to chloroform appear to be available (Chapter 5). A single controlled study has been performed. In this study, subjects were exposed to 70 or 178 mg of chloroform/day (≈1 or 2.5 mg/kg/day assuming 70 kg body weight) for at least 1 year (DeSalva et al., 1975). Neither liver function tests nor blood urea nitrogen determinations (a measure of kidney function) revealed statistically significant differences between exposed and control subjects. Case reports involving abuse of medicines containing chloroform (Wallace, 1950; Conlon, 1963) are not adequate for risk assessment because of the small numbers of patients, exposure to other agents, and imprecise estimates of intake.

Subchronic and chronic toxicity experiments with rats, mice, and dogs, when considered together (Table 5-4), do not clearly establish a NOAEL or NOEL. Although no adverse effects were observed in four strains of mice given 17 mg/kg/day of chloroform, 6 days/week for 2 years (Roe et al., 1979), at the lowest dose level tested (i.e., 15 mg/kg/day, 6 days/week for 7.5 years) in dogs, chloroform treatment was associated with an elevation in SGPT in some but not all of the other tested clinical chemistry indicies of hepatic damage (Heywood et al., 1979). The livers of dogs treated with chloroform at this dosage level had larger and more numberous "fatty cysts" than were found in controls. These fatty cysts consisted of aggregations of vacuolated histiocytes. No effect on survival, growth, organ weights, gross and histological appearance of other organs, or hematologic or urinalysis values was observed at this dosage level. Hence 15 mg/kg/day (6 days/week) represents a LOAEL for dogs for effects on the liver. Chronic oral administration of 60 mg/kg/day of chloroform (6 days/week) was associated with slight hepatic changes in rats (Palmer et al., 1979) and with increased incidences of moderate to severe renal disease in male mice of sensitive strains (Roe et al., 1979).

None of the three species tested in long-term experiments appeared to be markedly more sensitive to the toxicity of chloroform than any other; dogs may have been slightly more sensitive. There were considerable differences among strains of mice in the sensitivity of the males to chloroform nephrotoxicity, as had also been observed in acute toxicity experiments.

As would be expected, dosages that produced little or no histologic or clinical chemistry evidence of toxicity when given subchronically (15 and 30 mg/kg/day; rats, dogs) resulted in greater evidence of toxicity when given for longer periods of time (Palmer et al., 1979; Heywood et al., 1979). The response to chloroform in the long-term studies may have been modified by the presence or absence of intercurrent respiratory and renal disease, but no consistent pattern is obvious from an inspection of the data in Table 5-4.

5.5.6 Target Organ Toxicity. Target organs characteristic of the acute toxicity of chloroform are the central nervous system, liver, kidney, and heart. For chronic exposure to chloroform, characteristic target organs are the liver and kidney, and possibly the central nervous system. Some dose-response data are available for the toxicity of chloroform to the liver, kidney, and central nervous system; these data are summarized in Table 5-5 by target organ. The studies from which these data are drawn are discussed more fully elsewhere in Chapter 5, but a comparison on the basis of endpoint (target organ) was also considered to be useful.

Manifestations of liver damage include centrilobular necrosis, vacuolization, disappearance of glycogen, fatty degeneration and swelling (Groger and

Table 5-5

Target Organ Toxicity of Chloroform

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
liver	inhalation, acute (surgical anesthesia)	human	induction = 20,000-40,000 ppm x a few minutes, plus maintenance = 1500-15,000 ppm x variable duration	necrosis, fatty degeneration in some patients	NIOSH, 1974; Goodman and Gilman, 1980; Wood-Smith and Stewart, 1964
liver	inhalation, acute	mice	100 ppm x 4 hours, single exposure	fatty infiltration	Kylin et al., 1963
liver	inhalation, acute	mice	200 ppm x 4 hours, single exposure	necrosis, fatty infiltration, increase in SOCT	Kylin et al., 1963
liver	inhalation, chronic	rats	25 ppm, 4 hours/day, 5 days/week x 6 months	no effect	Torkelson et al., 1976
liver	inhalation, chronic	rats	25 ppm, 7 hours/day, 5 days/week x 6 months	lobular granular degeneration, focal necrosis	Torkelson et al., 1976
liver	inhalation, chronic	rats	50 or 85 ppm, 7 hours/day, 5 days/week x 6 months	marked centrilobular granular degeneration	Torkelson et al., 1976

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
liver	oral, acute	mice	30 mg/kg bw; single dose	fatty infiltration	Jones et al., 1958
liver	oral, acute	mice	133 mg/kg, single dose	massive fatty infiltration and severe necrosis	Jones et al., 1958
liver	oral, acute	mice	355 mg/kg, single dose	massive fatty infiltration and severe necrosis	Jones et al., 1958
liver	oral, acute	mice	60 mg/kg, single dose	no effect	Reitz et al., 1980
liver	oral, acute	mice	240 mg/kg, single dose	hepatocellular necrosis and swelling; inflammation	Reitz et al., 1980
liver	oral (drinking water) subchronic	rats	20, 38, 57, 81, or 160 mg/kg/day x 90 days	transient hepatosis (at 30 and 60, but not at 90 days)	Jorgenson and Rushbrook, 1980
liver	oral (drinking water) subchronic	mice	64 or 97 mg/kg/day x 90 days	transient centrilobular fatty change (at 30 and 60 but not at 90 days)	Jorgenson and Rushbrook, 1980

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
liver	oral (drinking water) subchronic	mice	145 or 190 mg/kg/day x 90 days	fatty change	Jorgenson and Rushbrook, 1980
liver	oral (gavage) subchronic	rats	410 mg/kg/day, 6 days/ week x 13 weeks	fatty change and necrosis	Palmer et al., 1979
liver	oral (capsule), subchronic	dogs	30 mg/kg/day, 7 days/ week x 13 weeks	no effect	Heywood et al., 1979
liver	oral (capsule), subchronic	dogs	45 mg/kg/day, 7 days/ week x 13 weeks	slight fatty change	Heywood et al., 1979
liver	oral (capsule), subchronic	dogs	60 mg/kg/day, 7 days/ week x 18 weeks	fatty degeneration, increase in SGOT and SGPT	Heywood et al., 1979
liver	oral (capsule), subchronic	dogs	120 mg/kg/day, 7 days/ week x 12 weeks	fatty degeneration, jaundice, increase in SGOT, SGPT, bilirubin	Heywood et al., 1979
liver	oral (gavage), chronic	rats	60 mg/kg/day, 6 days/ week x 80 weeks	minor histological changes and decrease in relative liver weight	Palmer et al., 1977
liver	oral (gavage), chronic	mice	60 mg/kg/day, 6 days/ week x 80 weeks	no effect	Roe et al., 1979

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
liver	oral (capsule), chronic	dogs	15 or 30 mg/kg/day, 6 days/week x 7.5 years	increases in SGPT and other serum indicators of hepatic damage, increase in size and number of fatty cysts (vacuolated histiocytes)	Heywood et al., 1979
liver	oral, chronic	humans	2.5 mg/kg/day for ≥ 1 year	no effect	De Salva et al., 1975
liver	dermal, acute	rabbits	3.98 g/kg x 24 hours under plastic cuff, single exposure	no macroscopic pathologic changes	Torkelson et al., 1976
kidney	inhalation, acute	mice, males of sensitive strains	5000 ppm, 1 hour, single exposure	necrosis and calcification of tubular epithelium	Deringer et al., 1953
kidney	inhalation, chronic	rats	25, 50, or 85 ppm, 7 hours/day, 5 days/week x 6 months	cloudy swelling of tubular epithelium	Torkelson et al., 1976
kidney	oral, acute	mice, males of sensitive strains	89 mg/kg, single dose	loss of glucose or protein in urine	Hill, 1978

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
kidney	oral, acute	mice, males of sensitive strains	149 mg/kg, single dose	loss of glucose or protein in urine	Hill, 1978
kidney	oral, acute	mice, male	15 mg/kg, single dose	no effect	Reitz et al., 1980
kidney	oral, acute	mice, male	60 mg/kg, single dose	focal tubular epithelial regeneration	Reitz et al., 1980
kidney	oral, acute	mice, male	240 mg/kg, single dose	severe diffuse cortical necrosis, focal tubular epithelial regeneration	Reitz et al., 1980
kidney	oral (drinking water) subchronic	rats	160 mg/kg/day x 90 days	no effect	Jorgenson and Rushbrook, 1980
kidney	oral (drinking water) subchronic	rats	≈300 mg/kg/day x 90 days	no effect	Chu et al., 1980b
kidney	oral (drinking water) subchronic	mice	290 mg/kg/day x 90 days	no effect	Jorgenson and Rushbrook, 1980

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
kidney	oral (capsule), subchronic	dogs	120 mg/kg/day, 7 days/week x 12 weeks	no effect	Jorgenson and Rushbrook, 1980
kidney	oral, chronic	humans	2.5 mg/kg/day, 7 days/week, for ≥1 year	no effect on BUN	De Salva et al., 1975
kidney	oral (gavage), chronic	rats	200 mg/kg/day, 5 days/week x 78 weeks	no effect	NCI, 1976
kidney	oral (gavage), chronic	mice	138 or 227 mg/kg/day, 5 days/week x 78 weeks	decreased incidence of renal disease	NCI, 1976
kidney	oral (gavage), chronic	mice, males of sensitive strains	17 mg/kg/day, 6 days/week x 80 weeks	no effect	Roe et al., 1979
kidney	oral (gavage) chronic,	mice, males of sensitive strains	60 mg/kg/day, 6 days/week x 80 weeks	increased incidence of moderate to severe renal disease	Roe et al., 1979

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
kidney	oral (gavage) chronic	mice, males of insensitive strains, females	60 mg/kg/day, 6 days/week x 80 weeks	no effect	Roe et al., 1979
kidney	oral (capsule), chronic	dog	15 mg/kg/day 6 days/week x 7.5 years	no effect	Heywood et al., 1979
kidney	oral (capsule), chronic	dog	30 mg/kg/day, 6 days/week x 7.5 years	increase in fat deposition in glomeruli	Heywood et al., 1979
kidney	dermal, acute	rabbits	1.0, 2.0, and 3.98 g/kg x 24 hours under plastic cuff, single exposure	degenerative changes in tubules	Torkelson et al., 1976
central nervous system (CNS)	inhalation, acute	humans	900-1400 ppm for $\geq$30 minutes, single exposure	dizziness, tiredness, headache	Lehmann and Hasegawa, 1910; Lehmann and Schmidt-Kehl, 1936
CNS	inhalation, acute	humans	4300-5100 ppm x 20 minutes, single exposure	dizziness, light intoxication	Lehmann and Hasegawa, 1910; Lehmann and Schmidt-Kehl, 1936

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
CNS	inhalation, acute	humans	1500-2000 ppm, single exposure	maintenance of light anesthesia (after induction)	Goodman and Gillman, 1980
CNS	inhalation, acute	humans	15,000 ppm, single exposure	maintenance of heavy anesthesia (after induction)	Goodman and Gillman, 1980
CNS	inhalation, acute	humans	20,000-40,000 ppm x a few minutes, single exposure	induction of anesthesia	NIOSH, 1974 Adriani, 1970
CNS	inhalation, acute	mice	2500 ppm x 12 hours, single exposure	no obvious effects	Lehmann and Flury, 1943
CNS	inhalation, acute	mice	3100 ppm x 1 hour, single exposure	light narcosis	Lehmann and Flury, 1943
CNS	inhalation, acute	mice	4100 ppm x 0.5 hours, single exposure	deep narcosis	Lehmann and Flury, 1943
CNS	inhalation, acute	cats	7200 or 21,500 ppm x 5 minutes, single exposure	disturbance of equilibrium	Lehmann and Flury, 1943
CNS	inhalation, acute	cats	7200 ppm x 60 minutes single exposure	light narcosis	Lehmann and Flury, 1943

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
CNS	inhalation, acute	cats	7200 ppm x 93 minutes single exposure	deep narcosis	Lehmann and Flury, 1943
CNS	inhalation, acute	cats	21,500 ppm x 10 minutes single exposure	light narcosis	Lehmann and Flury, 1943
CNS	inhalation, acute	cats	21,500 ppm x 13 minutes single exposure	deep narcosis	Lehmann and Flury, 1943
CNS	inhalation, chronic	humans	20-71 ppm (with excursions to 1163 ppm lasting 1.5-2 minutes) for 4-8 hours/day, 5 days/week	tiredness	Challen et al., 1958
CNS	inhalation, chronic	humans	77 to 237 ppm (with excursions to ≈1163 ppm lasting 1.5-2 minutes) for 4-8 hours/day, 5 days/week	tiredness, depression, occasional silliness or staggering during the workday	Challen et al., 1958

Table 5-5

Target Organ Toxicity of Chloroform (cont.)

Target Organ	Route and Type of Exposure	Species	Dose or Exposure	Effect on Target Organ	Reference
CNS	oral, acute	rats	350 mg/kg single dose	minimum narcotic dose (MND_{50})	Jones et al., 1958
CNS	oral (drinking water), subchronic	rats	20-160 mg/kg/day x 90 days	dose-related signs of depression during 1st week only	Jorgenson and Rushbrook, 1980
CNS	oral (drinking water), subchronic	mice	32-290 mg/kg/day x 90 days	dose-related signs of depression during 1st week only	Jorgenson and Rushbrook, 1980
CNS	oral (drinking water) subchronic	rats	≈300 mg/kg/day x 90 days	no histopathologic changes in brain	Chu et al., 1982b
CNS	oral (gavage), subchronic	rats	60 mg/kg/day 6 days/week x 80 weeks	no effect on gross or histologic appearance of brain	Palmer et al., 1977

Grey, 1979). In the kidney, chloroform exposure produces necrosis of the proximal and distal convoluted tubules (Eschenbrenner and Miller, 1945a). The mechanism by which chloroform produces these effects has been extensively studied in experimental animals. From the studies summarized in Section 5.3.1 (Brown et al., 1974; Ilett et al., 1973; Docks and Krishna, 1976; Ekström et al., 1981; Lavigne and Marchand, 1974; McMartin et al., 1981; Masuda et al., 1980; Harris et al., 1982; Stevens and Anders, 1981), it appears that chloroform is first metabolized in the target organ by microsomal drug metabolizing enzymes to a reactive intermediate, probably phosgene, which in turn can react by various pathways, depending on glutathione levels, one of which is the covalent binding to liver proteins resulting in necrotic lesions. A similar mechanism may or may not occur in the kidney (Kluwe and Hook, 1981).

5.5.7 Factors that Modify the Toxicity of Chloroform. Several substances alter the toxicity of chloroform, most probably by modifying the metabolism of chloroform to a reactive intermediate (see Section 5.4). These substances are of interest because humans may be accidentally or intentionally exposed to them. Factors that potentiate the toxic effects induced by exposure to chloroform include ethanol (Kutob and Plaa, 1962; Sato et al., 1980, 1981), polybrominated biphenyls (Kluwe and Hook, 1978), steroids (Clemens et al., 1979), and ketones (Hewitt et al., 1979; Jernigan and Harbison, 1982; Branchflower and Pohl, 1981). Disulfiram and its metabolites (Scholler et al., 1970; Masuda and Nakayama, 1982; Gopinath and Ford, 1975) and high carbohydrate diets (Nakajima et al., 1982), appear to protect against chloroform toxicity.

5. REFERENCES

Adriani, J. 1970. The Pharmacology of Anesthetic Drugs. Springfield, Illinois, Charles C. Thomas. pp. 57-60.

Bomski, H., A. Sobolweska and A. Strakowski. 1967. [Toxic damage of the liver by chloroform in chemical industry workers.] Arch. Gewerbepathol. Gewerbehy. 24: 127-134. (Ger.) (Cited in Utidjian, 1976; NIOSH, 1974.)

Bowman, F.J., J. Borzelleca and A.E. Munson. 1978. The toxicity of some halomethanes in mice. Toxicol. Appl. Pharmacol. 44: 213-215.

Branchflower, R.V. and L.R. Pohl. 1981. Investigation of the mechanism of the potentiation of chloroform-induced hepatotoxicity and nephrotoxicity by methyl, n-butyl ketone. Toxicol. Appl. Pharmacol. 61: 407-413.

Brown, B.R., Jr., I.G. Sipes and A.M. Sagalyn. 1974. Mechanisms of acute hepatic toxicity: Chloroform, halothane and glutathione. Anesthesiology. 41: 554-561.

Butler, T.C. 1961. Reduction of carbon tetrachloride *in vivo* and reduction of carbon tetrachloride and chloroform *in vitro* by tissues and tissue constituents. Jour. Pharmacol. Exp. Ther. 134: 311-319.

Challen, P.J.R., D.E. Hickish and J. Bedford. 1958. Chronic chloroform intoxication. Brit. Jour. Ind. Med. 15: 243-249.

Chu, I., V. Secours, I. Marino and D.C. Villeneuve. 1980. The acute toxicity of four trihalomethanes in male and female rats. Toxicol. Appl. Pharmacol. 52: 351-353.

Chu, I., D.C. Villenuve, V.E. Secours and G.C. Becking. 1982a. Toxicity of trihalomethanes: I. The acute and subacute toxicity of chloroform, bromodichloromethane, chlorodibromomethane and bromoform in rats. J. Environ. Sci. Health. B17: 205-224.

Chu, I., D.C. Villenuve, V.E. Secours, G.G. Becking and V.E. Valli. 1982b. Toxicity of trihalomethanes: II. Reversibility of toxicological changes produced by chloroform, bromodichloromethane, chlorodibromomethane and bromoform in rats. J. Environ. Sci. Health. B17: 225-240.

Cianflone, D.J., W.R. Hewitt, D.C. Villenueve and G.L. Plaa. 1980. Role of biotransformation in the alterations of chloroform hepatotoxicity produced by Kepone and Mirex. Toxicol. Appl. Pharmacol. 53: 140-149.

Clemens, T.L., R.N. Hill, L.P. Bullock, W.D. Johnson, L.G. Sulatos and E.S. Vesell. 1979. Chloroform toxicity in the mouse: Role of genetic factors and steroids. Toxicol. Appl. Pharmacol. 48: 117-130.

Conlon, M.F. 1963. Addiction to chlorodyne. Brit. Med. Jour. 2: 1177-1178.

Culliford, D. and H.B. Hewitt. 1957. The influence of sex hormone status on the susceptibility of mice to chloroform-induced necrosis of the renal tubules. Jour. Endocrinol. 14: 381-393.

Danni, O., O. Brossa, E. Burdino, P. Milillo and G. Ugazio. 1981. Toxicity of halogenated hydrocarbons in pretreated rats: An experimental model for the study of integrated permissible limits of environmental poisons. Int. Arch. Occup. Environ. Health. 49: 105-112.

Deringer, M.K., T.B. Dunn and W.E. Heston. 1953. Results of exposure of strain C3H mice to chloroform. Proc. Soc. Exp. Biol. Med. 83: 474-479.

DeSalva, S., A. Volpe, G. Leigh and T. Regan. 1975. Long-term safety studies of a chloroform-containing dentifrice and mouth-rinse in man. Food Cosmet. Toxicol. 13: 529.

Divincenzo, G.D. and W.J. Krasavage. 1974. Serum ornithine carbamyl transferase as liver response test for exposure to organic solvents. Amer. Indust. Hyg. Assoc. J. 35: 21-29.

Docks, E.L. and G. Krishna. 1976. The role of glutathione in chloroform-induced hepatotoxicity. Exp. Molec. Pathol. 24: 13-22.

Ekström, T., J. Hoegbers and B. Jernstroem. 1982. Inhibition of hepatic, gamma-glutamyl-cysteine synthetase by chloroform. Biochem. Pharmacol. 31: 3510-3513.

Eschenbrenner, A.B. and E. Miller. 1945a. Induction of hepatomas in mice by repeated oral administration of chloroform, with observations on sex differences. Jour. Natl. Canc. Instit. 5: 251-255.

Eschenbrenner, A.B. and E. Miller. 1945b. Sex differences in kidney morphology and chloroform necrosis. Science. 102: 302-303.

Goodman, L.S. and A. Gilman. 1980. The Pharmacological Basis of Therapeutics. 6th ed. New York, MacMillan and Co.

Gopinath, C. and E.J.H. Ford. 1975. The role of microsomal hydroxylases in the modification of chloroform hepatotoxicity in rats. Brit. Jour. Exp. Pathol. 56: 412-422.

Groger, W.K.L. and T.F. Grey. 1979. Effect of chloroform on the activities of liver enzymes in rats. Toxicology. 14: 23-28.

Harris, R.N., J.H. Ratnayake, V.F. Garry and M.W. Anders. 1982. Interaction hepatotoxicity of chloroform and carbon tetrachloride. Toxicol. Appl. Pharmacol. 63: 281-291.

Heller, H. and F.H. Smirk. 1932. The influence of anaesthetics and hypnotics on the absorption and excretion of water. Jour. Physiol. (London). 76: 292-302.

Hewitt, W.R., H. Mujajima, M.G. Côté and G.L. Plaa. 1980. Acute alteration of chloroform-induced hepato- and nephrotoxicity by n-hexane, methyl n-butyl ketone and 2,5-hexanedione. Toxicol. Appl. Pharmacol. 53: 230-248.

Hewitt, W.R., H. Miyajima, M.G. Côté and G.C. Plaa. 1979. Acute alteration of chloroform-induced hepato- and nephrotoxicity by Mirex and Kepone. Toxicol. Appl. Pharmacol. 48: 509-527.

Heywood, R., R.J. Sortwell, P.R.B. Noel, A.E. Street, D.E. Prentice, F.J.C. Roe, P.F. Wardsworth, A.N. Worden and N.J. Van Abbe´. 1979. Safety evaluation of toothpaste containing chloroform. III. Long-term study in beagle dogs. Jour. Environm. Pathol. Toxicol. 2: 835-851.

Hill, R.N. 1978. Differential toxicity of chloroform in the mouse. Ann. New York Acad. Sci. 298: 170-175.

Hjelle, J.J., A.S. Gordon and D.R. Peterson. 1982. Studies on carbon tetrachloride-ethanol interactions in mice. Toxicol. Lett. 10: 17-24.

IARC (International Agency for Research on Cancer). 1976. Disulfirams. In: Some Carbamates, Thiocarbamates and Carbazides. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. WHO: Lyon, France. IARC, p. 85-96.

Ilett, K.F., W.D. Reid, I.G. Sipes and G. Krishna. 1973. Chloroform toxicity in mice: Correlation of renal and hepatic necrosis with covalent binding of metabolites to tissue macromolecules. Exp. Molec. Pathol. 19: 215-229.

Jernigan, J.D. and R.D. Harbison. 1982. Role of biotransformation in the potentiation of halocarbon hepatotoxicity by 2,5-hexanedione. J. Toxicol. Environ. Health. 9: 761-781.

Jones, W.M., G. Margolis and C.R. Stephen. 1958. Hepatotoxicity of inhalation anesthetic drugs. Anesthesiology. 19: 715.

Jorgenson, T.A. and C.J. Rushbrook. 1980. Effects of chloroform in the drinking water of rats and mice: Ninety-day subacute toxicity study. SRI International, Menlo Park, California. Publication No. EPA-600/1-80-030. Available from National Technical Information Service, Springfield, Virginia (NTIS PB80-219108).

Kimura, E.T., D.M. Ebert and P.W. Dodge. 1971. Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol. Appl. Pharmacol. 19: 699-704.

Klaassen, C.D. and G.L. Plaa. 1966. Relative effect of various chlorinated hydrocarbons on liver and kidney function in mice. Toxicol. Appl. Pharmacol. 9: 139-151.

Kluwe, W.M. and J.B. Hook. 1978. Polybrominated biphenyl-induced potentiation of chloroform toxicity. Toxicol. Appl. Pharmacol. 45: 861-869.

Kluwe, W.M. and J.B. Hook. 1981. Potentiation of acute chloroform nephrotoxicity by the glutathione depletion diethyl maleate and protection by the microsomal enzyme inhibitor piperonyl butoxide. Toxicol. Appl. Pharmacol. 59: 457-466.

Knocker, P. and J. Mandelstam. 1944. Rhythmic changes in the rat produced by a single injection of chloroform. Nature (London). 154: 148-149.

Krantz, J.C., Jr. and C.J. Carr. 1965. Pharmacologic Principles of Medical Practice. 6th ed. Baltimore, Williams and Wilkins.

Krus, S. and Z. Zaleska-Rutczynski. 1970. Morphological counterparts of the genetically determined resistance of mice to chloroform. Experientia. 26: 101-102.

Kurtz, C.M., J.H. Bennett and H.H. Shapiro. 1936. Electrocardiographic studies during surgical anesthesia. J. Amer. Med. Assoc. 106: 434-441.

Kutob, S.D. and G.L. Plaa. 1962a. The effect of acute ethanol intoxication on chloroform-induced liver damage. Jour. Pharmacol. Exp. Teratol. 135: 245-251.

Kutob, S.D. and G.L. Plaa. 1962b. A procedure of estimating the hepatotoxicity potential of certain industrial solvents. Toxicol. Appl. Pharmacol. 4: 354.

Kylin, B., H. Reichard, I. Sumegi and S. Yllner. 1963. Hepatotoxicity of inhaled trichloroethylene, tetrachloroethylene and chloroform. Single exposure. Acta. Pharmacol. Toxicol. 20: 16-26.

Lavigne, J.G. and C. Marchand. 1974. The role of metabolism in chloroform hepatotoxicity. Toxicol. Appl. Pharmacol. 29: 312-326.

Lehmann, K.B. and F.F. Flury. 1943. Chlorinated hydrocarbons. In: Toxicology and Hygiene of Industrial Solvents, 1943. K.B. Lehmann and F.F. Flury, Eds. Williams and Wilkins Co., Baltimore. p. 138-145, 191-196.

Lehmann, K.B. and Hasewaga. 1910. [Studies of the absorption of chlorinated hydrocarbons in animals and humans.] Arch. Hyg. 72: 327-342. (Ger.) (Cited in NIOSH, 1974.)

Lehmann, K.B. and L. Schmidt-Kenl. 1936. [The thirteen most important chlorinated aliphatic hydrocarbons from the standpoint of industrial hygiene.] Arch. Hyg. 116: 131-200. (Ger.) (Cited in NIOSH, 1974.)

Lucas, G.H.W. 1928. A study of the fate and toxicity of bromine and chlorine containing anesthetics. Jour. Pharmacol. Exp. Ther. 34: 223.

Mansuy, D., P. Beaune, T. Cresteil, M. Lange and J.P. Lerous. 1977. Evidence for phosgene formation during liver microsomal oxidation of chloroform. Biochem. Biophys. Res. Commun. 79: 513-517.

Masuda, Y. and N. Nakayama. 1982. Protective effect of diethyldithiocarbamate and carbon disulfide against liver injury induced by various hepatotoxic agents. Biochem. Pharmacol. 31: 2713-2725.

Masuda, Y., I. Yano and T. Murano. 1980. Comparative studies on the hepatotoxic actions of chloroform and related halomethanes in normal and phenobarbital-pretreated animals. Jour. Pharmacobio. Dyn. 3: 53-64.

McLean, A.E.M. and E.K. McLean. 1969. Diet and toxicity. Brit. Med. Bull. 25: 278-281.

McLean, A.E.M. 1970. The effect of protein deficiency and microsomal enzyme induction by DDT and phenobarbitone on the acute toxicity of chloroform and a pyrrolizidiue alkaloid, retrorsine. Brit. Jour. Exp. Pathol. 51: 317-321.

McMartin, N.D., J.A. O'Connor, Jr., L.S. Kaminsky. 1981. Effects of differential changes in rat hepatic and renal cytochrome P-450 concentrations on hepatotoxicity and nephrotoxicity of chloroform. Res. Commun. Chem. Pathol. Pharmacol. 31: 99-110.

Nakajima, T., Y. Koyama and A. Sato. 1982. Dietary modification of metabolism and toxicity of chemical substances with specific reference to carbohydrate. Biochem. Pharmacol. 31: 1005-1011.

NCI (National Cancer Institute). 1976. Report on Carcinogenesis Bioassay of Chloroform. Available from National Technical Information Service, Springfield, Virginia. (NTIS PB-264-018).

NIOSH (National Institute for Occupational Safety and Health). 1974. Criteria for a Recommended Standard-Occupational Exposure to Chloroform. Available from National Technical Information Service, Springfield, Virginia. (NTIS PB-246-695).

Oettel, H. 1936. Arch. Exp. Pathol. Pharmacol. 183: 655.

Orth, O.S., R.R. Liebenow and R.T. Capps. 1951. III-The effect of chloroform on the cardiovascular system. In: R.M. Waters (Ed). Chloroform - A Study After 100 Years. University of Wisconsin Press, Madison, 1951. p. 39-75.

Palmer, A.K., A.E. Street, F.J.C. Roe, A.N. Worden and N.J. Van Abbe'. 1979. Safety evaluation of toothpaste containing chloroform. II. Long-term studies in rats. Jour. Environm. Pathol. Toxicol. 2: 821-833.

Paul, B.B. and D. Rubinstein. 1963. Metabolism of carbon tetrachloride and chloroform by the rat. Jour. Pharmacol. Exp. Ther. 141: 141-148.

Piersol, G.M., A.J. Tumen and L.S. Kau. 1933. Fatal poisoning following the ingestion of chloroform. Med. Clin. North Am. 17: 587-601.

Pohl, L.R., B. Bhooshan, N.F. Whittaker and G. Krishna. 1977. Phosgene: A metabolite of chloroform. Biochem. Biophys. Res. Commun. 79: 684-691.

Pohl, L.R. 1979. Biochemical toxicology of chloroform. Rev. Biochem. Toxicol. 1: 79-107.

Reid, W.D. and G. Krishna. 1973. Centrilobular hepatic necrosis related to covalent binding of metabolites of halogenated aromatic hydrocarbons. Exp. Molec. Pathol. 18: 80-95.

Reitz, R.H., J.F. Quast, W.T. Stott, P.G. Watanabe and P.J. Gehring. 1980. Pharmacokinetics and macromolecular effects of chloroform in rats and mice: Implications for carcinogenic risk estimation. Water Chlorinat. Environm. Impact Health Eff. 3: 983-993.

Reynolds, E.S. and A.G. Yee. 1967. Liver parenchymal cell injury. V. Relationships between patterns of chloromethane - ^{14}C incorporation into constituents of liver *in vivo* and cellular injury. Lab. Invest. 16: 591-603.

Roe, F.J.C., A.K. Palmer, A.N. Worden and N.J. Van Abbe´. 1979. Safety evaluation of toothpaste containing chloroform. I. Long-term studies in mice. Jour. Environm. Toxicol. 2: 799-819.

Sato, A., T. Nakajima and Y. Koyama. 1980. Effects of chronic ethanol consumption on hepatic metabolism of aromatic and chlorinated hydrocarbons in rats. Br. J. Indust. Med. 37: 382-386.

Sato, A., T. Nakajima and Y. Koyama. 1981. Dose-related effect of a single dose of ethanol on the metabolism in rat liver of some aromatic and chlorinated hydrocarbons. Toxicol. Appl. Pharmacol. 60: 8-15.

Sax, N.I. 1979. Dangerous Properties of Industrial Materials, Fifth edition. New York, Van Nostrand Reinhold Company, Inc. p. 193.

Scholler, K.L. 1966. Elektronmikroskopische untersuchungen an leberzellen der Ratte nach narkosen mit verschiedenen inhlalationsnarkotica. Der Anaesthetist. 15: 145-148. (Ger).

Scholler, K.L. 1967. Effect of haloethane and chloroform on the fine structure and protein synthesis of rat liver. Experimentia. 23: 652-655. (In German).

Scholler, K.L. 1970. Modification of the effects of chloroform on the rat liver. Brit. Jour. Anaesth. 42: 603-605.

Schroeder, H.G. 1965. Acute and delayed chloroform poisoning. Brit. J. Anaesth. 37: 972-975.

Sipes, I.G., G. Krishna and J.R. Gillette. 1977. Bioactivation of carbon tetrachloride, chloroform, and bromotrichloromethane: Role of cytochrome P-450. Life Sci. 20: 1541-1548.

Stevens, J.L. and M.W. Anders. 1981. Effect of cysteine, diethyl maleate, and phenobarbital treatment on the hepatoxicity of (1H)- and (2H)-chloroform. Chem. Biol. Interact. 37: 207-217.

Storms, W.W. 1973. Chloroform parties. J. Amer. Med. Assoc. 225: 160.

Torkelson, T.R., F. Oyen and V.K. Rowe. 1976. The toxicity of chloroform as determined by single and repeated exposure of laboratory animals. Amer. Ind. Hyg. Assoc. Jour. 37: 697-705.

Utidjian, H.M.D. 1976. Recommendations for a chloroform standard. Jour. Occupat. Med. 18: 253.

Van Dyke, R.A., M.B. Chenoweth and A.V. Poznak. 1964. Metabolism of volatile anesthetics. I. Conversion _in vivo_ of several anesthetics to $^{14}CO_2$ and chloride. Biochem. Pharmacol. 13: 1239-1247.

von Oettigen, W.F. 1964. The halogenated hydrocarbons of industrial and toxicological importance. Amsterdam, Elsevier.

Wallace, C.J. 1950. Hepatitis and nephrosis due to cough syrup containing chloroform. Calif. Med. 73: 442.

Watrous, W.M. and G.L. Plaa. 1972. Effect of halogenated hydrocarbons on organic ion accumulation by renal cortical slices of rats and mice. Toxicol. Appl. Pharmacol. 22: 528-543.

Winslow, S.G. and H.B. Gerstner. 1978. Health aspects of chloroform - A review. Drug Chem. Toxicol. 1: 259-275.

Wood, S., B. Wardley-Smith, M. J. Halsey and C.J. Green. 1982. Hydrogen bonding in mechanisms of anesthetic tested with chloroform and deuterated chloroform. Br. J. Anaesth. 54: 387-391.

Wood-Smith, F.G. and H.C. Stewart. 1964. Drugs in Anaesthetic Practice. Washington, D.C. Butterworth, Inc. pp. 131-135.